Agricultural Research Service

Red River Valley Agricultural Research Center

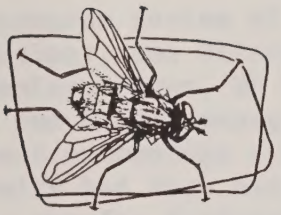
Fargo, ND

PLANT ENTOMOLOGY PROGRAM

RECOMMENDATIONS & STATUS REPORT

NOVEMBER 1990

National Program Staff



Preface

Prior to the plant entomology program review of the Insect Biochemistry, Insect Genetics and Cellular Biology, and Oilseeds Research Units, Red River Valley Agricultural Research Center, Fargo, ND held on August 14-16, 1989, discussions between NPS, Area, and Center management led to a number of observations concerning the plant entomology program. It was noted that in 1964 two entomology research units had been established in the Metabolism and Radiation Research Laboratory - one for fundamental research on the discovery and development of genetic approaches to insect control, including the use of radiation-induced sexual sterility; the other to explore aspects of insect growth and development to serve as the basis for the development of selective insecticides by the private sector. These two units were highly productive and they earned a very high prestige. In fact, over those 15 years or so the Metabolism and Radiation Research Laboratory became a "port-of-call" for leading and aspiring entomologists from all over the world. In 1987 the units were merged into one unit, namely the Insect Biochemistry and Molecular Genetics Research Unit with approximately 18 SY's reporting to one Research Leader (RL). In March of 1989, this unit was again split into two units, each containing nine scientists in order to distribute the administrative and management work load between two research leaders.

NPS feels that Fargo should be recognized as one of the Agency's premier locations devoted to excellence in molecular entomology and insect genetics. To ensure a continuing defined and clear mission, and to ensure that the two units are organized and staff effectively so as to continue to provide program impact in molecular entomology and insect control, additional strategic planning relative to molecular entomology was undertaken after the review, including program analysis and a Molecular Entomology workshop (see Appendix B). Assessments of the research program necessitated some changes in assignments of scientific, technical and support staff, in addition to program redirections and reallocation of resources among the CWU projects. This report documents the subject matter of the review held August 14-16, 1989, at Fargo and the interim actions, and details the NPS recommendations for the research thrusts and problems to be addressed by the plant entomology program (see Appendices A, B, D, E, and F and Sections IV and VI for further details).

Robert M. Faust
National program Leader
ARS National Program Staff

Center Overview

Mission:

The Red River Valley Agricultural Research Center oversees the research at three laboratories on the North Dakota State University campus at Fargo, ND: 1) the Biosciences Research Laboratory; 2) the Northern Crop Science Laboratory; and 3) the Hard Red Spring and Durum Wheat Quality Laboratory. Research at the BRL includes the metabolism and biochemistry of agricultural chemicals in plants and animals, and regulation, reproduction, and metabolism of biologically active chemicals in insects, reproduction and genetics of insects and screwworm research. The NCSL conducts research that helps expand and retain profitable production of barley, hard red spring wheat, durum, sunflowers, and sugarbeets. Work at the HRSDWQL is directed toward developing new methods of measuring wheat quality and monitoring quality of current production.

History:

Dr. Harold Flor, a plant pathologist, was the first ARS scientist to be located at Fargo in the early 1930's. Over the next 30 years, several additional ARS scientists were added in the areas of agronomy, plant pathology and chemistry and located in the respective University departments. They worked with flax, wheat, barley and sunflowers.

The Metabolism and Radiation Research Laboratory (now the Biosciences Laboratory) received its first planning funds in 1960 in response to national concerns about the use of chemicals in food production. The Laboratory opened in 1964 with Dr. R. C. Bushland as director. In 1983, Congressional funding provided for the construction of the new Northern Crop Science Laboratory which was occupied in May 1988. There are presently 126 total ARS employees, including 49 scientists, at the Center. Over the years, ARS has had numerous cooperative agreements with the Agricultural Experiment Station and University departments. Many of our scientists have Adjunct Professor appointments at the University.

From its inception, the BRL contained two entomological management units, Insect Genetics and Radiation Biology, and Insect Physiology and Metabolism. These two organizational units remained until 1987 when they were combined into one unit, named Insect Biochemistry and Molecular Genetics, containing 18 scientists. In March 1989, this unit was split into two units, Insect Biochemistry, and Insect Genetics and Cellular Biology, each containing nine scientists.

Don C. Zimmerman
Director

Center Overview

Table of Contents

		Page
Preface by Robert M. Faust		i
Center Overview by Don C. Zimmerman		ii
I.	EXECUTIVE SUMMARY	1
II.	Mission Statements of Research Units	2
III.	Objectives of the Plant Entomology Program Review	6
IV.	Current CWU Projects: Plant Entomology	7
	A. Insect Biochemistry	8
	B. Insect Genetics & Cellular Biology	18
	C. Oilseeds Research	31
V.	Summary of Major Contributions, Impact on Science & Impact on Technology	35
VI.	Recommendations/Status	45
	A. Insect Biochemistry	45
	B. Insect Genetics & Cellular Biology	46
	C. Oilseeds Research	52
VII.	Appendices	53
	A. Agenda & Participants of Entomology Program Review, August 14-16, 1989/Follow-up of Review, April 23-25, 1990/ Correspondence & Miscellaneous Materials Pertaining to Restructuring, 1989-90	54
	B. Workshop Recommendations for ARS Research on Molecular Entomology and Insect Genetics, ARS National Research Leaders' Meeting, April 10-13, 1990, Washington, D.C.	65
	C. SCA 58-82HW-8-7 on Genetic Sexing, Major Accomplishments	68
	D. Background, Workshop Recommendations, and Summary of Results of the Sterile Boll Weevil Program	70
	E. Communications Relative to Genetic Sterility in Heliothis	92
	F. Communications Relative to Russian Wheat Aphid Research	103
	G. Communications Relative to Coordinated Research to Determine the Geographic Origin of Heliothis spp. Dispersing from Indigenous Hosts and Habitats	110
	H. Poster Titles/Scientists, Entomology Program Review, August 14-16, 1989	114

Table of Contents (Cont.)

Page

I. USDA Research Priorities for Fiscal Year 1992 (Joint Council on Food and Agricultural Sciences) 117

J. Entomology Review - 1989 (separate volume upon request; document prepared by the Insect Biochemistry, Insect Genetics and Cellular Biology, and Oilseeds Research Units in preparation for the review)

Table of Contents (Cont.)

Page

I. EXECUTIVE SUMMARY

A plant entomology program review of the Insect Biochemistry, Insect Genetics and Cellular Biology, and Oilseeds Research Units was held on August 14-16, 1989, in Fargo, ND with a follow-up of the review made on April 23-25, 1990. The purpose of the review was to: (1) assess current research activities and objectives in relation to the ARS mission and its priorities, and assess the focus of the program, its relevance, progress, and its impact on ARS objectives; (2) identify researchable knowledge gaps; (3) define additional research activities in terms of the Laboratory's mission that can be accomplished with existing and/or recommended new resources; and (4) formulate recommendations for future research. Participants included representatives from the ARS National Program Staff (NPS), management, and scientists of the three research units, as well as collaborators. A decision was made by NPS that a full report of the program review and complete documentation of the recommendations would be delayed until a new research leader for the Insect Genetics and Cellular Biology Research Unit was appointed to fill the vacated position and a workshop was held to address the Agency's research program in molecular entomology and insect genetics with guidance from the NPS in the interim.

The Fargo plant entomology research program over the next 5 years is to focus on developing molecular and cellular approaches for the control and management of pest and beneficial insects. Ten broad areas of research emphasis that represent important continuing and/or high priority needs are recommended:

(1) Define the neuroendocrine role in regulating biochemical changes involved in reproduction and development.

(2) Determine the endocrine control of synthesis of cuticular waxes which function as pheromones and kairomones.

(3) Determine the biosynthesis and function of unique long-chain keto and methyl-branched alcohols in insect metamorphosis.

(4) Develop molecular genetics and DNA vectors for genetic engineering.

(5) Define bioregulation of insect reproduction and development.

(6) Develop cryopreservation methodology for insect germplasm.

(7) Define the genetics of natural insect populations.

(8) Develop breeding lines of sunflower with resistance or tolerance to insects.

(9) Determine insect-host plant interactions of sunflower pests.

(10) Determine the bionomics of major sunflower insect pests.

Pest insects to be addressed include Heliothis sp., cotton boll weevil, corn rootworm, Russian wheat aphid, sunflower head moth, banded sunflower moth, red sunflower seed weevil, stem weevil, cabbage looper, and the diamondback moth.

II. Mission Statements of Research Units

Research Units

Modecode: 5442-05-35

NORTHERN PLAINS AREA
FARGO, NORTH DAKOTA
RED RIVER VALLEY AGRICULTURAL RESEARCH CENTER
INSECT BIOCHEMISTRY RESEARCH

Address: BRL, PO BOX 5674-NDSU STATION
FARGO, ND 58105
FARGO NORTH DAKOTA 58105

Research Leader: DENNIS R. NELSON
SUPERVISORY RESEARCH CHEMIST

Telephone Number: (701)239-1286

Mission Statement:

The mission of the Insect Biochemistry Research Unit is to develop molecular approaches for the biorational control of insect pests. Study areas include: (1) define the neuroendocrine role in regulating biochemical changes involved in reproduction and development; (2) determine the endocrine control of synthesis of cuticular waxes which function as pheromones and kairomones; (3) determine the biosynthesis and function of unique long-chain keto and metnyl-branched alcohols in metamorphosis of lepidopteran pupae; (4) determine insect-host plant interactions of sunflower pests; (5) conduct basic screwworm research in support of APHIS.

Research Units

Modecode: 5442-05-15 NORTHERN PLAINS AREA
FARGO, NORTH DAKOTA
RED RIVER VALLEY AGRICULTURAL RESEARCH CENTER
INSECT GENETICS AND CELLULAR BIOLOGY

Address: BRL, PO BOX 5674-NDSU STATION
FARGO, ND 58105
FARGO NORTH DAKOTA 58105

Research Leader: SUDHIR KARL NARANG
SUPV. RES. GENETICIST

Telephone Number: (701)239-1270

Mission Statement:

The mission of the Insect Genetics and Cellular Biology Research Unit is to develop cellular and molecular approaches for the control and management of pest and beneficial insects. Research areas include: (1) Molecular Genetics and DNA vectors for genetic engineering, (2) bioregulation of insect reproduction and development, (3) cryopreservation of insect germplasm, and (4) the genetics of natural insect populations.

Research Units

Modecode: 5442-05-20 NORTHERN PLAINS AREA
FARGO, NORTH DAKOTA
RED RIVER VALLEY AGRICULTURAL RESEARCH CENTER
OILSEEDS RESEARCH

Address: NCSL, P.O. BOX 5677 UNIV. STA.
FARGO, ND 58105
FARGO NORTH DAKOTA 58105

Research Leader: BRADY A. VICK
SUPV. RES. CHEMIST

Telephone Number: 701-239-1310

Mission Statement:

The mission of the unit is to: (1) Reduce the cost of sunflower production by developing breeding lines of sunflower with improved yield potential, quality characteristics and resistance or tolerance to diseases and insects; (2) Collect and classify wild helianthus species and evaluate for traits; (3) Develop new cytogenetic methods for crossing wild helianthus species with H. Annuus; (4) Determine the role of oxygenated fatty acids in growth and differentiation of oil-seed crops; and (5) Determine the bionomics of the sunflower stem weevil, sunflower beetle, and banded sunflower moth.

III. Objectives of the Plant Entomology Program Review

1. Assess current research activities and objectives in relation to the ARS mission and its priorities, and assess the focus of the program, its relevance, progress, and its impact on ARS objectives.

2. Identify researchable knowledge gaps, if any.

3. Define additional research activities in terms of the ARS mission that can be accomplished with existing and/or recommended new resources.

4. Formulate recommendations that will make the program even better, and that will help guide the activities of line and staff officials for future research.

IV. Current CWU Projects: Plant Entomology

A. Insect Biochemistry

Agricultural Research Service
Research Management Information System
ARS Project System
11/09/90

Executive Summary Sheet

For ARS Project No. 5442-24000-017-00D
Accession Number 0143478
Mode Code 5442-05-35

Appropriated

EFFECT OF THE HOST-PLANT AND PHOTOPERIOD ON
INSECT REPRODUCTION

NORTHERN PLAINS AREA
FARGO, NORTH DAKOTA
RED RIVER VALLEY AGRICULTURAL RESEARCH CENTER
INSECT BIOCHEMISTRY RESEARCH

Project Status: ACTIVE Start: 04/13/90 Term: 04/17/95

Current Official Funding Levels:

Net to Location:	Permanent	Temporary
FY 90	$ 314,190	$ -205,018
FY 91	$ 314,190	$ 0

****** Official Strategic Plan Codes ******

2.4.01.1.c	40%
2.4.01.3.c	60%

Reason Project was Initiated: Normal Progression

Comments:
Replacing CRIS Projects #5442-24000-005-00D & 5442-24000-007-00D.
PERMANENT fund transfer of $314,190. Revised Project Statement on file in Area Office. NOTE:Change in 417 per NPL (M8G) 5/30/90

APPROVED:

Area Director: on 04/09/90 by T. J. Army
Assoc Depty Admin: on 04/13/90 by Robert R. Oltjen

Created: on 04/04/90 By M81
Last Modified: on 05/30/90 By M3G

Form AD-416 Document Printed 11/09/90 *****OFFICIAL PROJECT*****
USDA Date Modified: 05/30/90

RESEARCH PROJECT DESC.-RES. RESUME

1. Accession: 0143478 2-4. Agency ID: ARS 5442-05-35 5. Project Number: 5442-24000-017-00D 6. Status: A=ACTIVE

7. Title: EFFECT OF THE HOST-PLANT AND PHOTOPERIOD ON INSECT REPRODUCTION

8. Performing Organization: 0000 8749
AGRICULTURAL RESEARCH SERVICE

Responsible Organization: 9095 8739
NORTHERN PLAINS
AREA OFFICE
AGRICULTURAL RESEARCH SERVICE

City/State/County/Zip/Cong. Dist.
FARGO
NORTH DAKOTA 58105 01

City/State/Zip
FORT COLLINS
COLORADO 80526

12. Investigator(s):
BARKER J F
RIEMANN J G

16. Research Location on Campus
B = NO

Project Type:
17-1. D=Appropriated 17-2. = 18-4.
19-1. $314,190 19-2. 2.00 20. S
21. Facilities: A = FEDERALLY OWNED
22. Regional Project Number:
A: - - B: - -

24. OBJECTIVES:
Determine factors involved in feeding/oviposition of sunflower stem weevil Cylindrocopturus adspersus & in oviposition of sunflower banded head moth Cochylis hospes. Develop methods to assess seed damage by insects using computer video image analysis. Determine cellular mechanisms involved in passage of sperm bundles through basilar membrane in testes of lepidopteran pests of sunflowers & determine cellular mechanisms involved in sperm maturation & spermatophore formation in vas deferens.

25. APPROACH:
Develop bioassay methods for feeding and ovipositional plant attractant chemicals for sunflower pests. Isolate and identify active chemicals and verify structures by synthesis. Conduct cytological and chemical analysis of secretory material involved in sperm movement and maturation. Determine mechanisms of continuous light induced sterility.

27. KEYWORDS:
SUNFLOWER HELIANTHUS STEM WEEVIL CYLINDROCOPTURUS ADSPERSUS BANDED MOTH COCHYLIS HOSPES GYPSY MOTH LYMANTRIA DISPAR PHOTOPERIOD OVIPOSITION ATTRACTANT FEEDING

Recommended			Approved			Concurred	
Signature	A/D	Date	Signature			A/D	Date
RL: DENNIS R. NELSON	A	04/05/90	NPL1:	Robert M. Faust		A	04/15/90
LD: DON C ZIMMERMAN	A	04/06/90	NPL2:	Philip A. Miller		A	04/18/90
AD: T. J. Army	A	04/09/90	NPL3:				00/00/00
FAO:		00/00/00	BPMS:	Joseph S. Garbarino		A	05/29/90
			ADA:	Robert R. Oltjen		A	04/18/90

28. Award Date:	29. Start Date:	30. Termination Date:	Duration: (months)
00/00/00	04/18/90	04/17/95	060

Form AD-417 Document Printed 11/09/90 *****OFFICIAL PROJECT*****

1. Accession: 0143478 | 2-4. Mode Code: ARS 5442-05-35 | 5. Project Number: 5442-24000-017-00D | Date Last Modified: 05/30/90

				Net to Location
STP(s)	2.4.01.1.c	Genetic Blueprint-Cells	40%	125,676
	2.4.01.3.c	Plant-Insect Interactions	60%	188,514
			Total Net to Location:	$314,190

32.	Basic Research:	90%	282,771	
33.	Applied Research:	10%	31,419	
34.	Development Effort:	0%	0	
		Total Net to Location:	$314,190	

	Commodity Code	Activity Code	Research Problem Area Code	Field of Science Code	Prime %	Net to Location
36.	2500	4500	207	0112	45	141,386
37.	2500	4500	207	0214	40	125,676
38.	6500	4900	318	0412	15	47,129
				Total Net to Location:		$314,191

Class.	Code	Description	%	Net to Location
Commodity	2550	SUNFLOWER	85	267,062
Commodity	6510	INSECTS	15	47,129
			Total Net to Location:	$314,191

Activity	4527	GYPSY MOTH	15	47,129
Activity	4560	REPROD,GROWTH,DEV-INSECTS	35	109,967
Activity	4562	BEHAV MOD AGENTS-INSECTS	20	62,838
Activity	4564	INSECT/HOST INTERACTIONS	15	47,129
Activity	4940	REPROD,GROWTH,DEVELOPMENT	15	47,129
			Total Net to Location:	$314,192

Special	E1A1	ENERGY CROPS PRODUCTION	100	314,190
Special	N668946	SCS - SOIL CONSER SER	80	251,352
Special	SABI	INSECTS BIOLOGY & CONTROL	100	314,190
Special	WQBIC	BIOCONTROL	30	94,257
Special	XPR	ENVIRONMENTAL POLLUTION	100	314,190

* Note: Rounding may cause minor differences in calculated value compared to the actual Net to Location.

Agricultural Research Service
Research Management Information System
ARS Project System
11/09/90

Executive Summary Sheet

For ARS Project No. 5442-24000-022-00D
Accession Number 0144788
Mode Code 5442-05-35

Appropriated

HORMONE-NEUROHORMONE INTERACTIONS AFFECTING
DEVELOPMENT, DIAPAUSE, AND REPRODUCTION

NORTHERN PLAINS AREA
FARGO, NORTH DAKOTA
RED RIVER VALLEY AGRICULTURAL RESEARCH CENTER
INSECT BIOCHEMISTRY RESEARCH

Project Status: ACTIVE Start: 10/01/90 Term: 09/30/95

Current Official Funding Levels:

Net to Location:	Permanent	Temporary
FY 91	$ 350,948	$ 0

****** Official Strategic Plan Codes ******

2.4.01.2.a	60%
2.4.01.2.c	40%

Reason Project was Initiated: Normal Progression

Comments:
Replacing CWU 5442-24000-003-00D. Permanent fund transfer of $350,948.
Project statement & peer review on file in Area Office.

APPROVED:

Area Director: on 06/21/90 by T. J. Army
Assoc Depty Admin: on 06/22/90 by Waldemar Klassen

Created: on 06/15/90 By MBI
Last Modified: on 07/03/90 By DFC

Form AD-416 Document Printed 11/09/90 *****OFFICIAL PROJECT*****
USDA Date Modified: 07/03/90

RESEARCH PROJECT DESC.-RES. RESUME

1. Accession: 0144788 | 2-4. Agency ID: ARS 5442-05-35 | 5. Project Number: 5442-24000-022-00D | 6. Status: A=ACTIVE

7. Title: HORMONE-NEUROHORMONE INTERACTIONS AFFECTING DEVELOPMENT, DIAPAUSE, AND REPRODUCTION

8. Performing Organization: 0000 8749
AGRICULTURAL RESEARCH SERVICE

City/State/County/Zip/Cong. Dist.
FARGO
NORTH DAKOTA 58105 01

Responsible Organization: 9095 8739
NORTHERN PLAINS
AREA OFFICE
AGRICULTURAL RESEARCH SERVICE

City/State/Zip
FORT COLLINS
COLORADO 80526

12. Investigator(s):
ADAMS T S
BUCKNER J S
NELSON D R

16. Research Location on Campus: A = YES

Project Type: 13-4.
17-1. D=Appropriated 17-2. = 19-1. $350,948 19-2. 1.70 20. 91
21. Facilities: A = FEDERALLY OWNED
22. Regional Project Number:
A: - - B: - -

24. OBJECTIVES:
The objective of the research is to define the endocrine and neuroendocrine interactions with the neurosecretory cells, ovaries, fatbody and the epidermis that regulate biosynthesis, storage, and release of natural products that are essential to reproduction, development, and homeostasis. The occurrence of proteins, peptides, hydrocarbons, and hormones in these processes will be characterized and the identity of biologically active chemicals determined.

25. APPROACH:
A bioassay for EDNH will be developed in the housefly and production of ecdysteroids measured by RIA. The isolation of EDNH will be carried out by HPLC. Characterize EDNH receptor binding for development of potential control technology. Determine the effects of JH, ECD, EDNH and OH on vitellogenin metabolism and evaluate their potential for control technology Describe the role of the ovaries and hormones in reprogramming epidermal tissue involved the synthesis of the sex pheromone. Develop antibodies against the proteins of urate granules, isolate the RNA's involved in the protein synthesis, and develop probes for DNA to determine the role of ecdysteroids in gene expression. Determine the hormonal mechanisms controlling the storage and release of uric acid from cells.

27. KEYWORDS:
INSECTS MUSCA DOMESTICA HELICTHIS VIRESCENS MANDUCA SEXTA NEUROHORMONES PEPTIDES VITELLOGENIN URIC ACID PHEROMONE TOMATO HORMWORM CABBAGE LOOPER DIAMOND BACK MOTH

Recommended Signature	A/D	Date	Approved Signature	A/D	Date	Concurred Signature	A/D	Date
RL: DENNIS R. NELSON	A	06/16/90	NPL1: Robert M. Faust	A	06/22/90			
LD: DON C ZIMMERMAN	A	06/19/90	NPL2:		00/00/00			
AD: T. J. Army	A	06/21/90	NPL3:		00/00/00			
PAO:		00/00/00	BPMS: Joseph S. Garbarino	A	07/03/90			
			ADA: Waldemar Klassen	A	06/22/90			

28. Award Date:	29. Start Date:	30. Termination Date:	Duration: (months)
00/00/00	10/01/90	09/30/95	060

1. Accession: 0144788 | 2-4. Mode Code: ARS 5442-05-35 | 5. Project Number: 5442-24000-022-00D | Date Last Modified: 07/03/90

				Net to Location
STP(s)	2.4.01.2.a	Secretion, Metab-Hormones	60%	210,569
	2.4.01.2.c	Insect Neurobiology	40%	140,379
			Total Net to Location:	$350,948
32.	Basic Research:		100%	350,948
33.	Applied Research:		0%	0
34.	Development Effort:		0%	0
			Total Net to Location:	$350,948

	Commodity Code	Activity Code	Research Problem Area Code	Field of Science Code	Prime %	Net to Location
36.	6500	4900	318	0410	40	140,379
37.	6500	4900	318	0412	60	210,569
				Total Net to Location:		$350,948

Class	Code	Description	%	Net to Location
Commodity	6510	INSECTS	100	350,948
			Total Net to Location:	$350,948
Activity	4940	REPROD,GROWTH,DEVELOPMENT	30	105,284
Activity	4941	HORMONES	50	175,474
Activity	4960	BEHAVIOR-TAXIS,TROP,SUBST	20	70,190
			Total Net to Location:	$350,948
Special	E1D1	ENERGY MULTI-COMM PRODUCT	100	350,948
Special	PST1	FUNDAMENTAL BIOLOGY	100	350,948
Special	XPR	ENVIRONMENTAL POLLUTION	100	350,948

* Note: Rounding may cause minor differences in calculated value compared to the actual Net to Location.

Agricultural Research Service
Research Management Information System
ARS Project System
11/09/90

Executive Summary Sheet

For ARS Project No. 5442-24000-023-00D
Accession Number 0144805
Mode Code 5442-05-35

Appropriated

METABOLISM OF HYDROCARBONS, NOVEL ALCOHOLS, & WAX ESTERS DURING GROWTH, METAMORPHOSIS, DIAPAUSE & RE

NORTHERN PLAINS AREA
FARGO, NORTH DAKOTA
RED RIVER VALLEY AGRICULTURAL RESEARCH CENTER
INSECT BIOCHEMISTRY RESEARCH

Project Status: ACTIVE Start: 10/01/90 Term: 09/30/95

Current Official Funding Levels:

Net to Location:	Permanent	Temporary
FY 91	$ 302,160	$ 0

****** Official Strategic Plan Codes ******

2.4.01.1.a 100%

Reason Project was Initiated: Normal Progression

Comments:
Replacing CWU 5442-24000-004-00D. Permanent fund transfer of $302,160. Project statement and peer review on file in Area Office.

APPROVED:

Area Director: on 06/21/90 by T. J. Army
Assoc Depty Admin: on 06/22/90 by Waldemar Klassen

Created: on 06/18/90 By M81
Last Modified: on 07/03/90 By DFC

Form AD-416 Document Printed 11/09/90 *****OFFICIAL PROJECT*****
USDA Date Modified: 07/03/90

RESEARCH PROJECT DESC.-RES. RESUME

1. Accession: 0144805 2-4. Agency ID: ARS 5442-05-35 5. Project Number: 5442-24000-023-00D 6. Status: A=ACTIVE

7. Title: METABOLISM OF HYDROCARBONS, NOVEL ALCOHOLS, & WAX ESTERS DURING GROWTH, METAMORPHOSIS, DIAPAUSE & RE

8. Performing Organization: 0000 8749
AGRICULTURAL RESEARCH SERVICE

City/State/County/Zip/Cong. Dist.
FARGO
NORTH DAKOTA 58105 01

Responsible Organization: 9095 8739
NORTHERN PLAINS
AREA OFFICE
AGRICULTURAL RESEARCH SERVICE

City/State/Zip
FORT COLLINS
COLORADO 80526

12. Investigator(s):
NELSON D R
ADAMS T S
POMONIS J G
BUCKNER J S

16. Research Location on Campus: B = NO

Project Type:
17-1. D=Appropriated 17-2. = 18-4.
19-1. $302,160 19-2. 2.10 20. 91
21. Facilities: A = FEDERALLY OWNED
22. Regional Project Number:
A: - - B: - -

24. OBJECTIVES:
Characterize specific components of surface waxes that function as semiochemicals or can be used as taxonomic indicators, describe their biosynthetic pathway and role of endocrine system in regulating biosynthesis. Determine the biosynthetic pathways for novel long-chain keto-alcohols during diapause, and of novel long-chain methyl-branched alcohols during the pupal stage; investigate potential inhibitors. Determine how these pathways relate to those for fatty acid biosynthesis.

25. APPROACH:
Use biochemical, immunological, and radioisotope techniques to determine biosynthesis/storage/transport/function/metabolism of lipids, waxes, long-chain alcohols and keto-alcohols, and long-chain methyl-branched alcohols and hydrocarbons in diapausing and developing insects. Survey insect orders to determine the range of their occurrence. Identify, quantitate and determine the fate of novel waxes, alcohols and hydrocarbons using capillary gas chromatography-mass spectrometry, NMR, etc. Verify structures by chemical synthesis. Determine the source of propionate used for methyl branches and how the specific position of the methyl branch is controlled. Develop cell-free biosynthetic systems to isolate and characterize enyzme systems involved in the synthesis and metabolism of these compounds. Determine their biological activity by developing appropriate bioassays. Determine the regulation of the metabolism of these waxes, alcohols, and hydrocarbons by hormones and the availability of substrates.

27. KEYWORDS:
INSECTS METHYL BRANCHED ALKANES LONG CHAIN METHYL BRANCHED ALCOHOLS LONG CHAIN KETO ALCOHOLS LONG CHAIN KETO ALDEHYDES BIOSYNTHESIS SURFACE WAXES PUPAE DIAPAUSE

Recommended			Approved			Concurred		
Signature	A/D	Date	Signature			A/D	Date	
RL: DENNIS R. NELSON	A	06/18/90	NPL1:	Robert M. Faust		A	06/22/90	
LD: DON C ZIMMERMAN	A	06/19/90	NPL2:				00/00/00	
AD: T. J. Army	A	06/21/90	NPL3:				00/00/00	
PAO:		00/00/00	BPMS:	Joseph S. Garbarino		A	07/03/90	
			ADA:	Waldemar Klassen		A	06/22/90	

26. Award Date: 00/00/00 29. Start Date: 10/01/90 30. Termination Date: 09/30/95 Duration: (months) 060

1. Accession:	2-4. Mode Code:	5. Project Number:	Date Last Modified
0144805	ARS 5442-05-35	5442-24000-023-00D	07/03/90

STP(s)			Net to Location
2.4.01.1.a	Prot, Lipids, Carbohydrates	100%	302,160
	Total Net to Location:		$302,160
32.	Basic Research:	100%	302,160
33.	Applied Research:	0%	0
34.	Development Effort:	0%	0
	Total Net to Location:		$302,160

	Commodity Code	Activity Code	Research Problem Area Code	Field of Science Code	Prime %	Net to Location
36.	6500	4900	318	0412	100	302,160
				Total Net to Location:		$302,160

Class.	Code	Description	%	Net to Location
Commodity	6510	INSECTS	100	302,160
			Total Net to Location:	$302,160
Activity	4940	REPROD,GROWTH,DEVELOPMENT	40	120,864
Activity	4941	HORMONES	20	60,432
Activity	4970	CHEMICAL COMPOSITION	40	120,864
			Total Net to Location:	$302,160
Special	BT35	BIOTECH-INSECTS-MA	50	151,080
Special	E1D1	ENERGY MULTI-COMM PRODUCT	100	302,160
Special	PST1	FUNDAMENTAL BIOLOGY	100	302,160
Special	XPR	ENVIRONMENTAL POLLUTION	100	302,160

* Note: Rounding may cause minor differences in calculated value compared to the actual Net to Location.

B. Insect Genetics & Cellular Biology

Agricultural Research Service
Research Management Information System
ARS Project System
11/09/90

Executive Summary Sheet

For ARS Project No. 5442-22000-005-00D
Accession Number 0143113
Mode Code 5442-05-15

Appropriated

MOLECULAR GENETIC APPROACHES FOR THE
TRANSFORMATION OF PEST AND BENEFICIAL INSECTS

NORTHERN PLAINS AREA
FARGO, NORTH DAKOTA
RED RIVER VALLEY AGRICULTURAL RESEARCH CENTER
INSECT GENETICS AND CELLULAR BIOLOGY

Project Status: ACTIVE Start: 08/04/88 Term: 08/03/93

Current Official Funding Levels:

Net to Location:	Permanent	Temporary
FY 88	$ 240,264	$ 0
FY 89	$ 237,861	$ 0
FY 90	$ 249,376	$ 25,000
FY 91	$ 350,680	$ 0

****** Official Strategic Plan Codes ******

2.2.01.1.g	50%
2.2.05.1.a	50%

Reason Project was Initiated: Normal Progression

Comments:

APPROVED:

Area Director: on by
Assoc Depty Admin: on 10/25/90 by Darrell F. Cole

Created: on 07/19/88 By SRR
Last Modified: on 10/25/90 By MBG

Form AD-416 Document Printed 11/09/90 *****OFFICIAL PROJECT*****
USDA Date Modified: 10/25/90

RESEARCH PROJECT DESC.-RES. RESUME

1. Accession: 0143118 | 2-4. Agency ID: ARS 5442-05-15 | 5. Project Number: 5442-22000-005-00D | 6. Status: A=ACTIVE

7. Title: MOLECULAR GENETIC APPROACHES FOR THE TRANSFORMATION OF PEST AND BENEFICIAL INSECTS

8. Performing Organization: 0000 9749
AGRICULTURAL RESEARCH SERVICE

Responsible Organization: 9095 8739
NORTHERN PLAINS
AREA OFFICE
AGRICULTURAL RESEARCH SERVICE

City/State/County/Zip/Cong. Dist.
FARGO
NORTH DAKOTA 58105 01

City/State/Zip
FORT COLLINS
COLORADO 80526

12. Investigator(s):
HEILMANN L J
MCDONALD I C
NARANG S K

16. Research Location on Campus:
A = YES

Project Type:
13-4.
17-1. D=Appropriated 17-2. = 19-1. $350,680 19-2. 2.00 20. 91

21. Facilities: A = FEDERALLY OWNED

22. Regional Project Number:
A: - - B: - -

24. OBJECTIVES:
Develop a system of gene transfer in Coleoptera and/or Lepidoptera that can be used to introduce deleterious genes into populations either for direct control of the pest or to produce single-sex, laboratory-reared populations for release.

25. APPROACH:
Use molecular genetic methods to search for DNA vectors for gene transfer. Isolate, engineer, and clone genes that are suitable for transposition. Determine the stability of transposed genes and assess the ability of the transposed genes to be expressed at a useful level for control or genetic sexing.

27. KEYWORDS:
GENE TRANSFER DELETERIOUS GENES DNA VECTORS GENETIC SEXING GENE CLONING MOLECULAR GENETICS BIOCONTROL COLEOPTERA LEPIDOPTERA BOLL WEEVEIL DIABROTICA HELIOTHIS

Recommended			Approved			Concurred		
Signature	A/D	Date	Signature			A/D	Date	
RL:		00/00/00	NPL1:				00/00/00	
LD:		00/00/00	NPL2:				00/00/00	
AD:		00/00/00	NPL3:				00/00/00	
PAO:		00/00/00	BPMS:				00/00/00	
			ADA:	Darrell F. Cole		A	10/25/90	

28. Award Date: 00/00/00 | 29. Start Date: 08/04/88 | 30. Termination Date: 08/03/93 | Duration: (months) 060

Form AD-417 Document Printed 11/09/90 *****OFFICIAL PROJECT*****

1. Accession:	2-4. Mode Code:	5. Project Number:	Date Last Modified:
0143118	ARS 5442-05-15	5442-22000-005-00D	10/25/90

STP(s)			Net to Location
2.2.01.1.c	Genetic Informat - Insects	50%	175,340
2.2.05.1.a	Manipulation Insect Genes	50%	175,340
	Total Net to Location:		$350,680

32.	Basic Research:	50%	175,340
33.	Applied Research:	50%	175,340
34.	Development Effort:	0%	0
	Total Net to Location:		$350,680

	Commodity Code	Activity Code	Research Problem Area Code	Field of Science Code	Prime %	Net to Location
36.	1200	4500	204	0313	15	52,602
37.	1400	4500	207	0313	35	122,738
38.	2100	4500	207	0313	50	175,340
				Total Net to Location:		$350,680

Class	Code	Description	%	Net to Location
Commodity	1280	SWEETCORN	15	52,602
Commodity	1410	CORN	35	122,738
Commodity	2110	UPLAND COTTON	50	175,340
		Total Net to Location:		$350,680
Activity	4502	CORN ROOTWORM	25	87,670
Activity	4514	BOLL WEEVIL	50	175,340
Activity	4537	TOBACCO BUDWORM	25	87,670
		Total Net to Location:		$350,680
Special	BC16	BIO CONTROL-INSECTS-BCGE	100	350,680
Special	BT31	BIOTECH-INSECTS-NAPEM	60	210,408
Special	BT32	BIOTECH-INSECTS-DS	10	35,068
Special	BT33	BIOTECH-INSECTS-DPR	10	35,068
Special	BT34	BIOTECH-INSECTS-PCT	10	35,068
Special	BT35	BIOTECH-INSECTS-MA	10	35,068
Special	N668946	SCS - SOIL CONSER SER	50	175,340
Special	PST1	FUNDAMENTAL BIOLOGY	50	175,340
Special	PST2	NONPESTICIDAL CONTROL	50	175,340
Special	SAEI	INSECTS BIOLOGY & CONTROL	80	280,544

Agricultural Research Service
Research Management Information System
ARS Project System
11/09/90

Executive Summary Sheet

For ARS Project No. 5442-24000-006-00D
Accession Number 0141204
Mode Code 5442-05-15

Appropriated

THE GENETICS OF NATURAL INSECT POPULATIONS AND MODERN METHODS

NORTHERN PLAINS AREA
FARGO, NORTH DAKOTA
RED RIVER VALLEY AGRICULTURAL RESEARCH CENTER
INSECT GENETICS AND CELLULAR BIOLOGY

Project Status: ACTIVE Start: 03/31/86 Term: 03/31/91

Current Official Funding Levels:

Net to Location:	Permanent	Temporary
FY 86	$ 655,648	$ 0
FY 87	$ 655,647	$ 0
FY 88	$ 544,179	$ 0
FY 89	$ 538,737	$ 75,000
FY 90	$ 477,069	$ 0
FY 91	$ 193,330	$ 0

****** Official Strategic Plan Codes ******

2.4.01.4.a 100%

Reason Project was Initiated: Normal Progression

Comments:
Updating investigators in the 5442-05-15 unit due to change in RL.

APPROVED:

Area Director: on 07/27/90 by T. J. Army
Assoc Depty Admin: on by

Created: on 03/10/86 By SRR
Last Modified: on 07/27/90 By M05

Form AD-416 Document Printed 11/09/90 *****OFFICIAL PROJECT*****
USDA Date Modified: 07/27/90

RESEARCH PROJECT DESC.-RES. RESUME

1. Accession: 0141204 | 2-4. Agency ID: ARS 5442-05-15 | 5. Project Number: 5442-24000-006-00D | 6. Status: A=ACTIVE

7. Title: THE GENETICS OF NATURAL INSECT POPULATIONS AND MODERN METHODS

8. Performing Organization: 0000 8749
AGRICULTURAL RESEARCH SERVICE

Responsible Organization: 9095 8739
NORTHERN PLAINS
AREA OFFICE
AGRICULTURAL RESEARCH SERVICE

City/State/County/Zip/Cong. Dist.
FARGO
NORTH DAKOTA 58105 01

City/State/Zip
FORT COLLINS
COLORADO 80526

12. Investigator(s):
ROEHRDANZ R L

16. Research Location on Campus:
A = YES

Project Type:

17-1. D=Appropriated 17-2. = 18-4.
19-1. $193,330 19-2. 4.60 20. 81
21. Facilities: A = FEDERALLY OWNED
22. Regional Project Number:
A: - - B: - -

24. OBJECTIVES:
Determine the extent and importance of genetic variation in and among native insect populations (i.e., screwworms, boll weevils, Diabrotica, and possibly others) and assess how the variation could affect the success of modern insect control methods (genetic control, pheromone suppression, biological control, chemical control, and integrated programs).

25. APPROACH:
Karyotypes of screwworm and boll weevil populations will be analyzed, correlated with isozyme data, mitochondrial DNA analyses and chromosome banding studies. For the corn rootworm, classical genetic, isozyme, DNA restriction enzyme and immunological approaches will be used to study inter-and intraspecific variation.

27. KEYWORDS:
SCREWWORM BOLL WEEVIL CORN ROOTWORM CHROMOSOMAL POLYMORPHISM KARYOTYPE MITOCHONDRIAL DNA MONOCLONAL ANTIBODIES IMMUNOLOGY

Recommended			Approved			Concurred	
Signature	A/D	Date	Signature			A/D	Date
RL: S. KARL NARANG	A	07/25/90	NPL1:				00/00/00
LD: DON C ZIMMERMAN	A	07/27/90	NPL2:				00/00/00
AD: T. J. Army	A	07/27/90	NPL3:				00/00/00
PAO:		00/00/00	BPMS:				00/00/00
			ADA:				00/00/00

28. Award Date:	29. Start Date:	30. Termination Date:	Duration: (months)
00/00/00	03/31/86	03/31/91	060

Form AD-417 Document Printed 11/09/90 *****OFFICIAL PROJECT*****

1. Accession:	2-4. Mode Code:	5. Project Number:	Date Last Modified:
0141204	ARS 5442-05-15	5442-24000-006-00D	07/27/90

STP(s)			Net to Location
2.4.01.4.a	Genetic Variation-Nat Pop	100%	193,330
	Total Net to Location:		$193,330

32.	Basic Research:	70%	135,331
33.	Applied Research:	30%	57,999
34.	Development Effort:	0%	0
	Total Net to Location:		$193,330

	Commodity Code	Activity Code	Research Problem Area Code	Field of Science Code	Prime %	Net to Location
36.	1400	4500	207	0513	32	61,866
37.	2100	4500	207	0513	48	92,798
38.	6500	4900	318	0513	20	38,666
				Total Net to Location:		$193,330

Class.	Code	Description	%	Net to Location
Commodity	1410	CORN	32	61,866
Commodity	2110	UPLAND COTTON	48	92,798
Commodity	6510	INSECTS	20	38,666
		Total Net to Location:		$193,330
Activity	4514	BOLL WEEVIL	15	29,000
Activity	4536	SCREWWORM	15	29,000
Activity	4560	REPROD,GROWTH,DEV-INSECTS	50	96,665
Activity	4950	ECOLOGY & POPUL DYNAMICS	20	38,666
		Total Net to Location:		$193,331
Special	E1A1	ENERGY CROPS PRODUCTION	75	144,998
Special	E1B1	ENERGY LIVESTOCK PRODUCT	25	48,333
Special	N668946	SCS - SOIL CONSER SER	50	96,665
Special	PST1	FUNDAMENTAL BIOLOGY	50	96,665
Special	PST2	NONPESTICIDAL CONTROL	50	96,665
Special	SABI	INSECTS BIOLOGY & CONTROL	50	96,665
Special	XACU	ANIMAL CARE & USE	10	19,333

Agricultural Research Service
Research Management Information System
ARS Project System
11/09/90

Executive Summary Sheet

For ARS Project No. 5442-24000-015-00D
Accession Number 0145779
Mode Code 5442-05-15

Appropriated

BIOREGULATION OF INSECT CUTICLE FORMATION

NORTHERN PLAINS AREA
FARGO, NORTH DAKOTA
RED RIVER VALLEY AGRICULTURAL RESEARCH CENTER
INSECT GENETICS AND CELLULAR BIOLOGY

Project Status: ACTIVE Start: 10/01/89 Term: 09/30/94

Current Official Funding Levels:

Net to Location:	Permanent	Temporary
FY 90	$ 200,571	$ 1,623
FY 91	$ 362,866	$ 0

****** Official Strategic Plan Codes ******

2.4.01.1.b	40%
2.4.01.1.c	60%

Reason Project was Initiated: Normal Progression

Comments:

APPROVED:

Area Director: on by
Assoc Depty Admin: on 09/10/90 by Darrell F. Cole

Created: on 06/22/89 By M45
Last Modified: on 09/10/90 By M8G

Form AD-416 Document Printed 11/09/90 *****OFFICIAL PROJECT*****
USDA Date Modified: 09/10/90

RESEARCH PROJECT DESC.-RES. RESUME

1. Accession: 0145779 2-4. Agency ID: ARS 5442-05-15 5. Project Number: 5442-24000-015-00D 6. Status: A=ACTIVE

7. Title: BIOREGULATION OF INSECT CUTICLE FORMATION

8. Performing Organization: 0000 8749
AGRICULTURAL RESEARCH SERVICE

Responsible Organization: 9095 8739
NORTHERN PLAINS
AREA OFFICE
AGRICULTURAL RESEARCH SERVICE

City/State/County/Zip/Cong. Dist.
FARGO
NORTH DAKOTA 58105 01

City/State/Zip
FORT COLLINS
COLORADO 80526

12. Investigator(s):
STILES B
LEOPOLD R A
NARANG S K

16. Research Location on Campus:
A = YES

Project Type:
18-4.
17-1. D=Appropriated 17-2. = 19-1. $362,866 19-2. 1.70 20. 01
21. Facilities: A = FEDERALLY OWNED
22. Regional Project Number:
A: - - B: - -

24. OBJECTIVES:
To gain an understanding of the unique cellular and genetic mechanisms controlling growth and development of the insect exoskeleton so that the resulting information can be used to improve current pest control strategies by alleviating environmental contamination with toxic chemical pesticides.

25. APPROACH:
Both in vivo (cotton boll weevil) and in vitro (cockroach embryo-derived cell line) systems will be utilized to study the endocrine and genetic regulation of cuticle protein synthesis. Cloned DNA libraries will be generated and screened with mono- and polyclonal antibodies for cuticle protein-encoding DNA which will be used as probes. Regulation of microfibril orientation within pupal and adult boll weevil endocuticle will be examined in an attempt to correlate stage-specific changes in cuticle protein content with a particular chitin fiber framework.

27. KEYWORDS:
ANTHONOMOUS GRANIS COTTON BOLL WEEVIL CUTICLE PROTEINS ENDOCRINE AND GENETIC REGULATION FIBER ORIENTATION

Recommended			Approved			Concurred	
Signature	A/D	Date	Signature		A/D	Date
RL:		00/00/00	NPL1:			00/00/00
LD:		00/00/00	NPL2:			00/00/00
AD:		00/00/00	NPL3:			00/00/00
PAO:		00/00/00	BPMS:			00/00/00
			ADA:	Darrell F. Cole	A	09/10/90

28. Award Date: 00/00/00 29. Start Date: 10/01/89 30. Termination Date: 09/30/94 Duration: (months) 060

Form AD-417 Document Printed 11/09/90 *****OFFICIAL PROJECT*****

1. Accession: 0145779 | 2-4. Mode Code: ARS 5442-05-15 | 5. Project Number: 5442-24000-015-00D | Date Last Modified: 09/10/90

				Net to Location
STP(s)	2.4.01.1.b	Exoskeleton-Formation	40%	145,146
	2.4.01.1.c	Genetic Blueprint-Cells	60%	217,720
			Total Net to Location:	$362,866

32.	Basic Research:	80%		290,293
33.	Applied Research:	10%		36,287
34.	Development Effort:	10%		36,287
		Total Net to Location:		$362,867

	Commodity Code	Activity Code	Research Problem Area Code	Field of Science Code	Prime %	Net to Location
36.	2100	4500	207	0313	100	362,866
				Total Net to Location:		$362,866

Class.	Code	Description	%	Net to Location
Commodity	2110	UPLAND COTTON	50	181,433
Commodity	2120	LONG FIBER COTTON	50	181,433
			Total Net to Location:	$362,866
Activity	4514	BOLL WEEVIL	80	290,293
Activity	4549	OTHER INSECT SPECIES	20	72,573
			Total Net to Location:	$362,866
Special	IPMB	IPM BASIC RESEARCH	10	36,287
Special	PST1	FUNDAMENTAL BIOLOGY	50	181,433
Special	SABI	INSECTS BIOLOGY & CONTROL	50	181,433

	808	
	193	
	197	

Agricultural Research Service
Research Management Information System
ARS Project System
11/09/90

Executive Summary Sheet

For ARS Project No. 5442-21350-001-00D
Accession Number 0145778
Mode Code 5442-05-15

Appropriated

CRYOBIOLOGY AND THE PRESERVATION OF GERMPLASM OF INSECTS

NORTHERN PLAINS AREA
FARGO, NORTH DAKOTA
RED RIVER VALLEY AGRICULTURAL RESEARCH CENTER
INSECT GENETICS AND CELLULAR BIOLOGY

Project Status: ACTIVE Start: 10/01/89 Term: 09/30/94

Current Official Funding Levels:

Net to Location:	Permanent	Temporary
FY 90	$ 156,791	$ 0
FY 91	$ 177,031	$ 0

****** Official Strategic Plan Codes ******

2.1.02.1.c	70%
3.5.03.1.a	30%

Reason Project was Initiated: Normal Progression

Comments:

APPROVED:

Area Director: on by
Assoc Depty Admin: on 10/25/90 by Darrell F. Cole

Created: on 06/22/89 By M45
Last Modified: on 10/25/90 By MBG

Form AD-416 Document Printed 11/09/90 *****OFFICIAL PROJECT*****
USDA Date Modified: 10/25/90

RESEARCH PROJECT DESC.-RES. RESUME

1. Accession: 0145778 2-4. Agency ID: ARS 5442-05-15 5. Project Number: 5442-21350-001-00D 6. Status: A=ACTIVE
7. Title: CRYOBIOLOGY AND THE PRESERVATION OF GERMPLASM OF INSECTS
8. Performing Organization: 0000 8749
AGRICULTURAL RESEARCH SERVICE

Responsible Organization: 9095 8739
NORTHERN PLAINS
AREA OFFICE
AGRICULTURAL RESEARCH SERVICE

City/State/County/Zip/Cong. Dist.
FARGO
NORTH DAKOTA 58105 01

City/State/Zip
FORT COLLINS
COLORADO 80526

12. Investigator(s):
ROJAS R R
LEOPOLD R A

16. Research Location on Campus: A = YES

Project Type:
17-1. D=Appropriated 17-2. = 18-4.
19-1. $177,031 19-2. 1.70 20. 81
21. Facilities: A = FEDERALLY OWNED
22. Regional Project Number:
A: - - B: - -

24. OBJECTIVES:
The objective of this project is to develop a technology for cryogenic storage of insect germplasm using knowledge gained by studying how insects naturally survive low temperatures and freezing.

25. APPROACH:
A variety of model insect species representing dipteran, lepidopteran and coleopteran pests will be surveyed for cold and/or freeze tolerance within developmental stages and for special strategies for cold temperature survival. Once the most cold tolerant stage is established, cryogenic storage methodology will be developed by optimizing cooling/warming rates, storage temperatures, cryoprotectant systems and environmental acclimation.

27. KEYWORDS:
MUSCA DOMESTICA COCHLIOMYIA HOMINIVORAX HOMEOSOMA ELECTELLUM DIABROTICA SSP. CRYOGENIC STORAGE OVERWINTERING ANTHONOMOUS GRANDIS

Recommended			Approved			Concurred		
Signature	A/D	Date	Signature		A/D	Date		
RL:		00/00/00	NPL1:			00/00/00		
LD:		00/00/00	NPL2:			00/00/00		
AD:		00/00/00	NPL3:			00/00/00		
PAO:		00/00/00	BPMS:			00/00/00		
			ADA:	Darrell F. Cole	A	10/25/90		

28. Award Date: 00/00/00 29. Start Date: 10/01/89 30. Termination Date: 09/30/94 Duration: (months) 060

Form AD-417 Document Printed 11/09/90 *****OFFICIAL PROJECT*****

1. Accession:	2-4. Mode Code:	5. Project Number:	Date Last Modified:
0145778	ARS 5442-05-15	5442-21350-001-000	10/25/90

				Net to Location
STP(s)	2.1.02.1.c	Arthropod Control	70%	123,922
	3.5.03.1.a	Biochem, Physiology-Insect	30%	53,109
			Total Net to Location:	$177,031

32.	Basic Research:	40%	70,812
33.	Applied Research:	30%	53,109
34.	Development Effort:	30%	53,109
		Total Net to Location:	$177,030

	Commodity Code	Activity Code	Research Problem Area Code	Field of Science Code	Prime %	Net to Location
36.	6700	4500	207	0414	75	132,773
37.	6800	4500	210	0414	25	44,258
				Total Net to Location:		$177,031

Class	Code	Description	%	Net to Location
Commodity	6710	MULTIPLE CROPS	75	132,773
Commodity	6810	MULTIPLE ANIMAL SPECIES	25	44,258
			Total Net to Location:	$177,031
Activity	4502	CORN ROOTWORM	10	17,703
Activity	4514	BOLL WEEVIL	10	17,703
Activity	4530	HOUSEFLY	25	44,258
Activity	4536	SCREWWORM	20	35,406
Activity	4549	OTHER INSECT SPECIES	10	17,703
Activity	4569	BIOCHEM & PHYSIOL-INSECT	25	44,258
			Total Net to Location:	$177,031
Special	BC12	BIO CONTROL-INSECTS-BCA	50	88,516
Special	IPMB	IPM BASIC RESEARCH	50	88,516
Special	N548805	NIH - NATL INST HEALTH	25	44,258
Special	PST1	FUNDAMENTAL BIOLOGY	60	106,219
Special	PST2	NONPESTICIDAL CONTROL	40	70,812

C. Oilseeds Research

Agricultural Research Service
Research Management Information System
ARS Project System
11/09/90

Executive Summary Sheet

For ARS Project No. 5442-24000-002-00D
Accession Number 0141946
Mode Code 5442-05-20

Appropriated

HOST-PLANT RESISTANCE AND MANAGEMENT SYSTEMS FOR
INSECT PESTS OF SUNFLOWER IN THE NORTHERN PLAINS

NORTHERN PLAINS AREA
FARGO, NORTH DAKOTA
RED RIVER VALLEY AGRICULTURAL RESEARCH CENTER
OILSEEDS RESEARCH

Project Status: ACTIVE Start: 04/07/87 Term: 04/06/92

Current Official Funding Levels:

Net to Location:	Permanent	Temporary
FY 87	$ 78,543	$ 0
FY 88	$ 112,133	$ 0
FY 89	$ 111,012	$ 0
FY 90	$ 109,482	$ 811
FY 91	$ 109,482	$ 0

****** Official Strategic Plan Codes ******

2.4.01.3.c 100%

Reason Project was Initiated: Normal Progression

Comments:

APPROVED:

Area Director: on by
Assoc Depty Admin: on 01/26/90 by Darrell F. Cole

Created: on 03/23/87 By SRR
Last Modified: on 01/26/90 By MBG

Form AD-416 Document Printed 11/09/90 *****OFFICIAL PROJECT*****
USDA Date Modified: 01/26/90

RESEARCH PROJECT DESC.-RES. RESUME

1. Accession: 0141946 | 2-4. Agency ID: ARS 5442-05-20 | 5. Project Number: 5442-24000-002-00D | 6. Status: A=ACTIVE

7. Title: HOST-PLANT RESISTANCE AND MANAGEMENT SYSTEMS FOR INSECT PESTS OF SUNFLOWER IN THE NORTHERN PLAINS

8. Performing Organization: 0000 8749
AGRICULTURAL RESEARCH SERVICE

Responsible Organization: 9095 8739
NORTHERN PLAINS
AREA OFFICE
AGRICULTURAL RESEARCH SERVICE

City/State/County/Zip/Cong. Dist.
FARGO
NORTH DAKOTA 58105 01

City/State/Zip
FORT COLLINS
COLORADO 80526

12. Investigator(s):
CHARLET L D
ZIMMERMAN D C

16. Research Location on Campus: A = YES

Project Type:
17-1. D=Appropriated 17-2. = 18-4.
19-1. $109,482 19-2. 1.10 20. 91
21. Facilities: D = STATE
22. Regional Project Number:
A: - - B: - -

24. OBJECTIVES:
To reduce sunflower crop losses and cost of sunflower pest control in the Northern Plains region by identifying insect resistant germplasm and natural enemies as part of a management system for the banded sunflower moth, Cochylis hospes, and the sunflower stem weevil, Cylindrocopturus adspersus.

25. APPROACH:
Parasitoid fauna and rates of parasitism in cultivated sunflower will be documented. Seasonal abundance, behavior, host stages attacked, and parasitization rate of the parasitoids will be studied in the field and the biology determined in the laboratory. Germplasm sources including cultivated material, interspecific crosses, and wild (native Helianthus spp.) will be evaluated for insect resistance in field nurseries. Promising germplasm will be incorporated into breeding lines with the assistance of ARS geneticists. Resistant germplasm will be analyzed to determine the mechanism of resistance.

27. KEYWORDS:
SUNFLOWER HELIANTHUS INSECTS PARASITOIDS NATURAL ENEMIES GERMPLASM BIOLOGY ENTOMOLOGY PEST MANAGEMENT HOST PLANT RESISTANCE BIOLOGICAL CONTROL BEHAVIOR

Recommended			Approved			Concurred	
Signature	A/D	Date	Signature			A/D	Date
RL:		00/00/00	NPL1:				00/00/00
LD:		00/00/00	NPL2:				00/00/00
AD:		00/00/00	NPL3:				00/00/00
PAO:		00/00/00	BPMS:				00/00/00
			ADA:	Darrell F. Cole		A	01/26/90

28. Award Date: 00/00/00 | 29. Start Date: 04/07/87 | 30. Termination Date: 04/06/92 | Duration: (months) 060

Form AD-417 Document Printed 11/09/90 ******OFFICIAL PROJECT******

1. Accession:	2-4. Mode Code:	5. Project Number:		Date Last Modified
0141946	ARS 5442-05-20	5442-24000-002-00D		01/26/90
STP(s) 2.4.01.3.c	Plant-Insect Interactions		100%	Net to Location 109,482
		Total Net to Location:		$109,482
32. Basic Research:			40%	43,793
33. Applied Research:			60%	65,689
34. Development Effort:			0%	0
		Total Net to Location:		$109,482

	Commodity Code	Activity Code	Research Problem Area Code	Field of Science Code	Prime %	Net to Location
36.	2500	4500	207	0412	100	109,482
				Total Net to Location:		$109,482

Class	Code	Description	%	Net to Location
Commodity	2550	SUNFLOWER	100	109,482
			Total Net to Location:	$109,482
Activity	4549	OTHER INSECT SPECIES	100	109,482
			Total Net to Location:	$109,482
Special	N668946	SCS - SOIL CONSER SER	100	109,482
Special	PST2	NONPESTICIDAL CONTROL	100	109,482
Special	SAIN	INSECT TOLERANT VARIETIES	100	109,482

V. Summary of Major Contributions, Impact on Science & Impact on Technology

MAJOR CONTRIBUTIONS OF INSECT BIOCHEMISTRY UNIT	IMPACT ON SCIENCE	IMPACT ON TECHNOLOGY
1968-1981---First to show that developing fly ovary produces a hormone (oostatic hormone) that regulates cyclical egg production. Developed hypothesis to explain how hormone interacts with other endocrines to regulate oogenesis. Because hormone inhibits action of other hormones, it has potential use as a pest control agent.	Stimulated scientists to look for oostatic hormones in other insects which were found in Rhodnius, Musca, & Aedes. Insect Reproduction Lab, Beltsville, isolating hormone from Musca & Univ. of Florida group has partially purified hormone from Aedes.	Until material is chemically characterized it will not impact.
1974-1979---Developed physiological aging technique based on degree of ovarian maturation that is more precise than chronological age for physiological studies.	Enabled mating, pheromone production, endocrine release and mating behavior to be correlated within and between species (i.e., eye gnats, houseflies, screwworms).	Used by APHIS & ARS to monitor age of screwworms attracted to hosts, baits & roosting sites. Determine age structure & ovipositional history of field population; wild flies vs. factory released, & efficiency of bait formulations in attracting different segments of population.
1984-1989---Ecdysteroids turn on pheromone biosynthesis.	New role for ecdysteroids; Male has genetic potential to make pheromone if proper hormones present.	Findings too new for impact.
1988-1989---JH plus ecdysteroids produce vitellogenin in females and males.	Males have genetic potential to make female compounds if proper hormones are present.	Findings too new for impact.
1972-1973---Metabolism & GLC analysis of the chemosterilant busulfan in boll weevil.	Established mass spectral data and interpretation of spectra of dimethanesulfonates. Developed method to analyze for dimethanesulfonates by GLC.	Used by ARS to get approval to run pilot boll weevil eradication program. Used at boll weevil Gast rearing facility to analyze diet & weevils in the eradication program.

1970---Discovered multiple-methyl-branched hydrocarbons in insects.	Stimulated work world-wide to characterize insect hydrocarbons and determine their uniqueness to insects, biosynthesis and function.	Provided the basis for chemical taxonomy.
1970-1989---Developed hypothesis to identify long-chain methyl-branched hydrocarbons and their mixtures from their mass spectra.	Changed the concepts of how hydrocarbons fragmented and what structural features were directing fragmentation.	Changed manner in which mass spectral data was published; hydrocarbons used for chemical taxonomy. Sex pheromones: 1978-G. morsitans; 1984-G. pallidipes. APHIS monitors factory & wild screwworms.
1982---Showed methyl branches in insect hydrocarbons are derived from propionate instead of acetate; Propionate converted to acetate by pathway found in plants.	Stimulated much work on lipid biosynthesis in arthropods. Other organisms use acetate for their methyl-branches, i.e., in farnesol, sterols.	Findings too new for impact.
1984---Discovered long-chain keto-alcohols and keto-aldehydes in surface lipids of diapausing pupae.	Stimulated interest in biosynthesis and in role of these lipids in survival of diapause pupae.	Findings too new for impact.
1984---Discovered acetate, acetoacetate & hydroxybutyrate esters of long-chain keto-alcohols in surface lipids of diapausing pupae.	Stimulated interest in biosynthesis and role of these unique esters, and in fate of ketone bodies in insects.	Findings too new for impact.
1989---Discovered very long-chain methyl-branched alcohols in lepidopteran pupae.	Stimulated interest in a new field in insect biochemistry.	Findings too new for impact.
1980-1989---Characterized uric acid & its storage granules as major nitrogen excretory product of Lepidoptera; showed it was both stored and released by fat body and epidermal cells - exact process determined by levels of ecdysteroids and JH.	Showed new physiological processes under endocrine regulation and stimulated interest in mechanisms of how hormones change functions of the cell.	Findings too new for impact.

Found a mating stimulant contact pheromone in surface lipids of female screwworm.	Findings not yet published.	Impact awaits compounds identification.
1967-1971---Synthesized aziridine chemosterilants & determined kinetics of decomposition.	New structures equal to traditional compounds; decomposition dependent on buffer concentration.	Recommended means of destroying aziridine compounds in toxic clean-up.
1984-1989---Colonized sunflower insect pests and developed assays for feeding and ovipositional attractants.	C. adspersus (stem weevil) & C. hospes (banded moth) now available for research year-round.	Video image analysis developed to measure feeding damage to sunflowers.

MAJOR CONTRIBUTIONS OF THE UNIT	IMPACT ON SCIENCE	IMPACT ON TECHNOLOGY
Boll Weevil Genetics and Sterilization		
Discovered gut cell death to be cause of radiation induced life shortening.	Had wide ranging entomological as well as medical implications.	Targeted the inability to obtain competitive sterile males for erradiacation program.
Prescribed correct irradiation procedure to obtain sterile males.		Used in present sterile-male boll weevil control program.
Ellucidated effect of diflubenzuron on chitin synthesis and female sterility.	Advanced method of studying chitin synthesis.	Technique to sterilize females released in sterile weevil releases. Used in present control program.
Determined heritability of various important traits in boll weevil.	Added significantly to quantitative genetics knowledge.	Foundation to a better mass rearing program.
Developed superior strain to use in sterile-male release control program.	Added significantly to quantitative nature of selection.	Greatly increase efficiency of released sterile male as well as reducing the cost of releasing and producing weevils.
Developed method to ameliorate the radiation-death syndrome by altering pre-irradiation diet.	Gave insight into role of radiation induced gut cell lesions in radioprotection, sensitivity and overall radiation syndrome.	Provides new methods to minimize radiation life shortening and loss of sexual competitiveness in the sterile male release boll weevil strain.
Radiation & Chemical Sterilization		
Correlated chemical structure of the mutagen to the induced genetic event.	Gave insight into why closely related chemical mutagens were not always near equal in mutagenic effectiveness.	Provided the method to produce highly efficient chemosterilants.
Showed that the use of chemical sterilents was not an efficient or safe means of sexually sterilizing insects.	Terminated this type of insect sterility research.	

MAJOR CONTRIBUTIONS OF THE UNIT	IMPACT ON SCIENCE	IMPACT ON TECHNOLOGY
Proved that the ratio of centromere length to arm length characterized lepidopteran, dipteran and hemipteran chromosomes.	Brought into question the hypothesis that holokinetic chromosomes conferred radio-resistance.	
Characterized the spermatogenic processes for several insects of economic importance.	Provided a data base for future studies.	
Demonstrated that insect chitin containing cuticle was produced in vitro and are sensitive to hormone control.	Provided a method of examining cuticle disposition in vitro.	Provided a means of screening cuticle synthesis inhibitors and growth regulators.
Enhanced activity of cuticle synthesis inhibitor in boll weevil.	Showed a synergism could exist between an insect growth regulator and an insect hormone.	Provided a means of extending the efficiency of diflurobenzuron.

MAJOR CONTRIBUTIONS OF THE UNIT	IMPACT ON SCIENCE	IMPACT ON TECHNOLOGY
Biosystematics.	Demonstrated use of biochemical, chromosomal, molecular, or more classical techniques for elucidating relationships of pest complexes.	1) Resolved questions regarding the postulated existence of screwworm variant types in support of the Mexican and Central American eradication effort. 2) Provided essential clarifications of corn rootworm pest complexes in regions of range overlap, clinal variation, or intergrade.
Isozyme studies of corn rootworms.	a. Biosystematic clarifications. b. Characterization of systems influenced by age, sex, or diet of adults.	1) Data on enzyme expression valuable to workers using isozyme profiles for genetic relatedness studies. 2) Provided a basis for studies of genetic regulatory mechanisms. 3) Demonstrated correlations between ovarian development and energy producing enzyme expression systems. 4) Provided new opportunities for research on reproductive biology at molecular & biochemical levels.

MAJOR CONTRIBUTIONS OF THE UNIT	IMPACT ON SCIENCE	IMPACT ON TECHNOLOGY
Constructed male-only housefly strains.	First insect strains of this type using manipulations of sex-determination & lethal factors.	Worldwide interest in developing similar strains for use in sterile-male control programs. Example: screwworms, mosquitoes, medfly, etc.
Characterization of male & female autosomal sex determiners in house flies.	a. Revised concept of sex-determination in species. b. Raised awareness of similar determiners in other species. c. Demonstrated geographic variation in patterns of sex-determiners.	1) Japanese, Italian, and American workers have used information in modeling studies of the evolution of sex-determination. 2) Used in studies of relations between geographic variation and pesticide usage patterns.
Formal genetics of house flies.	Established house fly as an important test organism for genetic research at morphological, physiological or molecular levels.	Provided genetic stocks and data on house flies to researchers throughout world for use in biological or genetic research. Example: pesticide resistance, endocrine research.
Genetic Control mechanisms in housefly.	a. Demonstrated methods for recovering transformations, inversion, compound autosomes, conditional lethals, meiotic drive factors, etc. b. Models of genetic control developed and cage tested.	1) Among first field tests of genetic control strains of insects. 2) Demonstrated many of the problems and pitfalls associated with genetic control.

JOURNAL OF RESEARCH of the National Bureau of Standards

Vol. 71C, No. 3, July–September 1967

Contents

Page

Mechanical Properties of Polymers

Mechanical properties of polymers: Introduction to the symposium. J. D. Ferry (University of Wisconsin, Madison, Wis.) 177

MAJOR CONTRIBUTIONS OF THE UNIT	IMPACT ON SCIENCE	IMPACT ON TECHNOLOGY
Cloning and characterizing of genes for separation of males and female insects.	Demonstrated for the first time that a protamine gene could be isolated from invertebrates. Also, identified a boll weevil vitelloprotein gene.	Offers a new method of studying 95% of all animal life. Make mass rearing of single sexes a reality.
Demonstrated that restriction enzyme analysis is an effective tool for studying population dynamics in the screwworm fly and the boll weevil.		Offers a powerful forensic measure for determining the source of an insect infestation.
Insect Cryopreservation & Overwintering Physiology	Defined the barriers to invertebrate cryopreservation and devised a generalized decision process for approaching cryopreservation of invertebrates, providing a framework for research in this field.	Redefined the cold tolerance and potential overwintering range of the sunflower moth, Homoeosoma electellum.
Combined Nuclear Magnetic Resonance Imaging & Spectroscopy for study developmental dynamics of insects.	Opened a new avenue of physical and chemical research for studying small organisms.	Demonstrated that the solenoid coil was a useful accessory for NMR near real time imaging and spectroscopy of small organisms

MAJOR CONTRIBUTIONS OF THE UNIT	IMPACT ON SCIENCE	IMPACT ON TECHNOLOGY
Developed & Perfected F_1 sterility. Proved success in controlling natural populations with lab., cage, and isolated field tests.		Used internationally to control commercial pests. Example: codling moth, fig moth, pests of stored grain. Considered today for cotton and corn isect control in U.S.
Proved chromosomal basis for sterility.	Proved chromosomal segregation in translocations different in holokinetic species.	
Proved sex-distortion due to recessive sex linked lethals.	One of few sex linked lethals in holokinetic chromosomes.	Provides additional method of obtaining sterility to control insect pests.
Elucidated role of apyrene and eupyrene sperm in reproduction.	Focused attention on sperm type and function in animal species.	Provides additional method in insect control.
Recorded on film embryo development of normal and those arising from irradiated sperm; as well as a unique technique for filming sperm transfer in the moth.	Provided scientific and educational material that was distributed world wide.	

VI. Recommendations/Status

A. Insect Biochemistry Research Unit

General comments:

The Insect Biochemistry Research Unit's plant entomology program over the next 5 years is to be focused on developing a fundamental base of insect molecular and cellular biology for use in molecular approaches to biorational control of major insect pests; major thrusts include studies on host-plant and photoperiod effects on insect reproduction, hormone/neurohormone interactions that affect insect development, diapause and reproduction, and insect metabolism of hydrocarbons, novel alcohols and wax esters during growth, metamorphosis, diapause and reproduction. Emphasis is to be placed on the house fly and the tobacco hornworm as representative model systems; other pest insects to be addressed include the sunflower head moth, the banded sunflower moth, the stem weevil, and Heliothis virescens. As resources can be made available, select vegetable insect pests such as the cabbage looper and the diamondback moth are to be added to the unit's focus.

Problems to be addressed, benefits and specific objectives recommended:

1. Host-plant and photoperiod effects on insect reproduction.

   Inadequate knowledge of the biology and ecology of pest and beneficial insects within variable ecosystems impedes the development of improved control strategies; methods and inadequate knowledge of the chemical and biological mechanisms of plant-insect interactions prevents rapid discovery and exploitation of effective and ecologically-selective methods of control. Research aimed at filling these gaps should aid in the development of environmentally-compatible insect pest control and help decrease crop losses (Barker, Riemann).

   a. Determine factors involved in feeding/oviposition of the sunflower stem weevil, Cylindrocopturus adsperus and factors involved in oviposition of the sunflower banded head moth, Cochylis hospes; develop methods for assessing seed damage by specific insects using computer video image analysis.

   b. Determine cellular mechanisms involved in the passage of sperm bundles through the basilar membrane in the testes of Lepidoptera pests of sunflowers and determine the cellular mechanisms involved in the maturation of sperm and spermatophore formation in the vas deferens of lepidopteran pests of sunflowers.

o Implementation status - implemented April 18, 1990

2. Hormone-neurohormone interactions affecting insect development, diapause and reproduction/insect metabolism of hydrocarbons, novel alcohols and wax esters during growth, metamorphosis, diapause and reproduction.

Lepidopteran pests are a major cause of crop loss and pesticide use; Dipteran pests are major vectors of disease and losses to the livestock industry. There is a need for the discovery, characterization and role of discrete semiochemicals involved in development, reproductive behavior and diapause, as well as with the hormonal regulation of their biosynthesis. Many pests survive from year to year by diapausing during periods of adverse conditions when they need protection against desiccation and pathogens, and during which they must handle metabolic waste products by means other than excretion. Also, specific components of the surface waxes of insects are involved in their behavior and in their attractiveness to parasites and predators. The ability to manipulate the biosynthesis or action of the semiochemicals involved in all these processes could be used to develop new methods of biorational pest control that are safer to the environment and ground-water sources (Nelson, Pomonis, Adams, Buckner).

a. Characterize specific components of insect surface waxes that function as pheromones, mating stimulants and kairomones, or taxonomic indicators.

b. Determine the role of the endocrine system in controlling the biosynthesis of biologically-active components of the cuticular waxes.

c. Determine the biosynthetic pathways unique to insects for the synthesis of long-chain keto-alcohols, long-chain methyl-branched alkanes and the novel very long-chain methyl-branched alcohols, and investigate potential inhibitors; Investigate substrate availability and other mechanisms that control the enzymic systems responsible for biosynthesis of the long-chain alcohols and alkanes and determine the interrelations between these pathways and those responsible for the synthesis of long-chain fatty acids.

o Implementation status - implemented October 1, 1990

B. Insect Genetics & Cellular Biology Research Unit

General Comments:

The Insect Genetics and Cellular Biology Research Unit's plant entomology program over the next 5 years is to be focused on developing molecular and cellular approaches for the control and management of pest and beneficial insects with major thrusts on molecular genetics and DNA vectors for genetic engineering, molecular bioregulation of insect reproduction and development, genetics of natural insect populations, and cryopreservation of insect germplasm. Emphasis is to be placed on major insect pests that include Heliothis sp., boll weevil, corn rootworm, and the Russian wheat aphid (as resources can be made available, see Appendix F). For the cryopreservation research effort, model systems that include housefly, screwworm fly, boll weevil, western and northern corn rootworms and sunflower moth will be used.

Problems to be addressed, benefits, and specific objectives recommended:

1. Molecular genetics and DNA vectors.

Genetic engineering technology has the potential to provide economical, safe and highly selective means of insect pest control through manipulation of the insect's own genes. Long-term use of insecticides has resulted in increasing resistance among target species and serious environmental contamination, especially contamination of the underground water table by these chemicals (approximately 40-45 million pounds of soil pesticides are used annually to control corn rootworms). There is a need to investigate all potential alternatives to chemical insecticides. In addition to control of pest insects, genetic engineering also has great potential for beneficial insects. For instance, using the same methods it should be possible to make these insects resistant to specific insecticides, or make them more cold hardy, therefore increasing their useful range.

The potential benefits of this long-range research program are i) compilation of a body of knowledge about the molecular genetics and genome structure of insects, ii) development of a technique for genetic transformation of major pest insects, iii) development from theory to practice of genetic sex ratio management using genetic engineering technology, and iv) expansion of the use of a proven safe and effective method of insect control, sterile male release.

The project team will expand molecular genetic studies of the boll weevil, Anthonomus grandis, in order to satisfy the long-term goal of developing new molecular methods of control generally applicable to other insects such as Diabrotica spp. and Heliothis spp. The overall objective will be to develop a system of gene transfer in Coleoptera and/or Lepidoptera that can be used to introduce deleterious genes into populations either for direct control of the pest or to produce single-sex, laboratory-reared populations for release.

As resources can be made available, the unit should initiate studies to control corn rootworm by developing molecular antisense RNA technology which will disrupt reproductive and olfactory processes. This would include genetically engineering one or more endogenous microorganisms (e.g., spiroplasm, bacteria, rickettsia) which can be introduced into native populations and result in disruption of the tubulin gene of sperm development or the antennae esterase gene for olfactory response (Heilmann, McDonald, Narang).

a. Isolate insect genes for proteins which either determine the sex of the insect or are expressed in a sex-specific manner, or which have potential use for genetic sex-ratio management.

b. Characterize the expression of isolated genes, sequence them and their control regions, and map them to the chromosomes by in situ hybridization and restriction fragment length polymorphisms.

c. Characterize the genome of the boll weevil, measuring the haploid DNA content of cells, relative amounts of highly repetitive, middle repetitive and single copy DNA and the nature of the arrangement of the repetitive and single copy DNA within the genome.

d. Identify and isolate transposable elements from A. grandis using cloned genes to screen different laboratory strains and geographic isolates for restriction fragment polymorphisms caused by insertion of DNA.

e. Determine the stability of transposed genes and assess the ability of the transposed genes to be expressed at a useful level for control of genetic sexing.

f. Determine the role of latent hereditary viruses in the backcross sterility phenomenon of Heliothis; determine the prevalence of hereditary viruses within the genus Heliothis and related genera; isolate and characterize Heliothis hereditary viruses. If hereditary viruses are shown to be the cause of inherited sterility in H. virescens backcross males, the viruses will be incorporated into H. zea males to try and induce sterility into the species. In any event, validate this phenomenon (see Appendix E); if viruses are not involved, identify and characterize the genes involved with backcross sterility in Heliothis as they relate to genetic sexing.

g. Determine the genetic mechanisms involved with insecticide resistance in Heliothis sp.; develop a gene library for Heliothis sp.

h. Delineate the involvement of sex-limited enzyme systems with reproduction or sensory perception in the corn rootworm; compare mechanisms of gene expression and regulation between the sexes of corn rootworm; identify DNA fragments from genomic or cDNA libraries containing sequences for enzyme production.

i. Develop antisense RNA technology to reduce corn rootworm development (as resources can be made available).

2. Genetics of natural insect populations.

In spite of the major efforts on research in support of nonchemical approaches to management of such insects as Heliothis, boll weevil and Russian wheat aphid, crops such as cotton and vegetables are still routinely treated on a field-by-field basis when the insects attain a specified density or damage level. Area-wide management of major insect pests will depend, in part, on information such as the origin and contribution of migrants to

the local pest population dynamics. Thus, it is exceedingly important to have the means to determine the impact of long-range insect pest migrants versus endemic insects on the overall population dynamics of the pest in a production area. Research addressing long-range insect migration and mesoscale movement have been identified by ARS as a critical need.

The overall objective of the project will be to determine the type and extent of genetic differences among natural insect populations (tobacco budworm, cotton bollworm, boll weevil and possibly several other insect pests or beneficial insects where appropriate) and assess how genetic composition of natural populations could affect the success of control methods. A multiple methological approach is to be used to identify genetic differences among target and source populations. The isozyme and DNA RFLP data is to be correlated with hydrocarbon data generated by the laboratory to determine the possible physiological basis of host specificity. Major emphasis will be placed on Heliothis sp. (Roehrdanz, Narang).

a. Determine the genetic composition of populations of H. virescens and H. zea (and boll weevil) with emphasis on genetic fingerprinting of natural populations.

b. Determine diagnostic allozyme, DNA RFLP and cuticular hydrocarbon markers for identification of populations and individuals of Heliothis sp. (and boll weevil).

c. Determine genetic and physiological differences among populations of Heliothis sp. (and boll weevil) collected from cultivated and wild host plants.

d. Determine the origin and contribution of migrants on Heliothis sp. (and boll weevil) population dynamics using isozyme frequency and RFLP data (see Appendix G).

e. Identify mitochondrial DNA variants that can be used as molecular markers to distinguish parasites and/or predators of the Russian wheat aphid that have diverse Old World geographic origins (as resources can be made available).

Note: Field validation of the Fargo casanova boll weevil strain competitiveness has been completed; the strain has been transferred to Mississippi State, MS (see Appendix D).

o Implementation status - project statement under preparation for review; target date for full implementation is April 1, 1991.

3. Molecular bioregulation of insect growth and development.

Since the advent of mechanized farming the American farmer has become increasingly dependent on the use of chemical insecticides to control insect pests. However, the farmers' arsenal of insecticides has been depleted by the occurrence of insect resistance, monumental costs for research and development and

environmental concerns. While this reliance on a single control method will hopefully become substantially reduced by substituting promising cultural and biological control methods, some form of chemical insecticides will continue to be the only method available for control of certain insect pests. For these reasons, it is appropriate and desirable that ARS continue to take the lead role in developing environmentally safer, pest-specific insecticides. This role can be achieved by conducting research programs that expose those vital life functions of insect which show a vulnerability to possible exogenous chemical control.

Insect cuticle formation is a critical developmental process which in recent years has received much attention, especially in the development of chitin synthesis inhibitors as possible insecticides. However, before they can be widely used in an agricultural or forest environment, much needs to be learned about the mode of action of these chemicals, their side affects, synergistic and antagonistic responses, degradation, build-up of insect resistance, etc. Unfortunately, the answers to some of these questions will remain unattainable until more is learned about basic development and growth of the cuticle. Further, the formulation of more effective and pest-specific cuticle synthesis inhibitors is limited by the lack of information on cuticle formation.

Although chitin is a common component of all insect cuticles, the diversity in construction of the exoskeletal body wall that is generally observed between the various stages of insect growth and between species is related to its association with the other components of the cuticle. The other major component of insect cuticle is protein (ca. 50% by dry weight). Protein with its many possible configurations is the obvious choice for contributing to inter- and intraspecific variation in the framework of insect cuticle. Three levels of the bioregulation of protein deposition in the cuticle of an insect will be studied: cellular (epidermal-directed pattern formation), endocrine and gene expression.

The primary insect to be used for these studies will be the cotton boll weevil. This insect is an excellent choice for such studies since little work has been conducted with cuticle proteins of coleopterous insects and none with a major agricultural pest. Also, preliminary evidence shows that there is a stage-specific change in the type of cuticle deposited and in the quality of proteins synthesized in this insect. The chitin synthesis inhibitor, diflubenzuron, is currently being used in pilot test programs to augment the sexual sterilization of boll weevil females for release as a possible control method. Thus, the information gained from these studies should be of interest to scientists, industry and to action agencies (Stiles, Leopold, Narang).

a. Develop an understanding of the unique molecular, cellular and genetic mechanisms regulating development of the insect exoskeleton.

b. Characterize insect cuticular proteins using polyacrylamide gel electrophoresis and poly and monoclonal antibodies with emphasis on the boll weevil.

c. Generate a cDNA library for the developmental stages of the boll weevil for use as a probe to identify cells which synthesize cuticular proteins and for delineating the effects of various insect hormones on the synthesis of cuticular proteins (this will allow for increased understanding of cuticle synthesis and regulation).

d. Develop an in vitro system for studying cuticle protein synthesis and regulation; develop an assay system capable of screening candidate biological control agents aimed at the insect cuticle, where possible.

o Implementation status - implemented October 1, 1989

4. Cryopreservation of insect germplasm.

Collections, evaluations, and germplasm preservation of insect pests, beneficial parasites and predators, for arthropod control are inadequate to meet the needs of crop protection strategies. Except for isolated cases, most insect germplasm preservation can be accomplished only through continuous rearing. Having the capability to halt genetic change by placing the insects in a cryopreserved state would eliminate some of the problems concerned with mass rearing of insects used in biocontrol programs and would allow stockpiling of insects. Cryogenic techniques, coupled with new methods in cell culture and gene modification of insects or insect cells, will facilitate the development of improved insect strains and the ability to stockpile genetically-defined strains (Rojas, Leopold).

a. Determine the optimum conditions for survival of insects using cryopreservation methodology, i.e., determine critical parameters such as cooling rate, type and concentration of cryoprotectant and cryoprotectant penetration, removal, and warming rate.

b. Analyze freezing injuries by the use of light and electron microscopy as well as differential scanning calorimetry and in vitro culture.

c. Determine possible effects of cryopreservation on longevity, reproduction and heredity of surviving insects and their progency.

d. Develop specialized techniques for handling insect eggs and embryos prior to freezing.

o Implementation status - implemented October 1, 1989

C. Oilseeds Research Unit

General Comments:

The Oilseeds Research Unit as related to plant entomology is to focus on developing breeding lines of sunflower with resistance or tolerance to insect pests and determining the bionomics of the sunflower stem weevil, sunflower beetle, banded sunflower moth, and the red sunflower seed weevil for use in strategies to control these pests.

Problems to be addressed, benefits, and specific objectives recommended:

1. Host-plant resistance and bionomics of sunflower insect pests.

   There is a need to reduce sunflower crop losses and the cost of sunflower pest control in the Northern Plains region. Emphasis is placed on the banded sunflower moth, sunflower stem weevil, sunflower beetle, and the red sunflower seed weevil. Completion of research in this area should allow the development of environmentally-compatible insect pest control and decrease losses to sunflower crops (Chalet).

   a. Identify and evaluate sunflower germplasm for insect resistance.

   b. Identify natural enemies of sunflower pests for use as part of a management system in conjunction with host-plant resistance.

   c. Continue cryobiology collaborative efforts with the Insect Genetics and Cellular Biology Research Unit.

o Implementation status - ongoing project which will terminate April 6, 1992.

VII. APPENDICES

APPENDIX A. Agenda & Participants of Entomology Program Review, August 14-16, 1989/ Follow-up of Review, April 23-25, 1990/ Correspondence & Miscellaneous Materials Pertaining to Restructuring, 1989-90

AGENDA FOR 1989 FARGO ENTOMOLOGY PROGRAM REVIEW

August 13		Robert Faust, NPL arrives in Fargo
August 14	0800 - 0830	Introduction & Opening Remarks by Don Zimmerman, CD; Robert Faust, NPL and Eldean Gerloff, AAD
	0830 - 0850	Overview of Project by Research Leaders G. Gassner
	0850 - 0910	D. Nelson
	0910 - 0920	B. Vick
	0920 - 0930	Break
	0930 - 1030	Reports
	1030 - 1130	Posters
	1130 - 1230	Lunch
	1230 - 1410	Reports
	1410 - 1425	Break
	1425 - 1605	Posters
	1605 - 1700	Return to Hotel
	1700 - 2130	RRVARC Picnic
August 15	0830 - 0950	Reports
	0950 - 1010	Break
	1010 - 1130	Posters
	1130 - 1300	Lunch
	1300 - 1330	Visit NCRL Entomology Laboratory L. Charlet
	1330 - 1340	Return to BRL from NCRL
	1340 - 1500	Reports
	1500 - 1515	Break
	1515 - 1615	Posters
	1615 - 1800	Outline Draft of Report - CD, NPL, RLs
	1800 - 2000	Dinner
August 16	0800 - 1200	Follow up Dialog with Scientists - NPL
	1200 - 1330	Lunch
	1330 - 1630	Overview of RRVARC, Including visit to Screwworm Research Group - NPL, AAD, CD

PARTICIPANTS

Insect Biochemistry Research Unit

Dennis R. Nelson, Research Leader
James S. Buckner, Research Chemist
J. George Pomonis, Research Chemist
John G. Riemann, Research Entomologist
Terrance S. Adams, Research Entomologist
John F. Barker, Research Entomologist

Insect Genetics & Cellular Biology Research Unit

George Gassner, Research Leader
Maurice E. Degrugillier, Research Entomologist
Odell A. Johnson, Entomologist
Roger A. Leopold, Research Entomologist
Ian C. McDonald, Geneticist Insects
David T. North, Research Geneticist Insects
Richard L. Roehrdanz, Research Geneticist Insects
Robert R. Rojas, Research Biologist
Brad Stiles, Research Biologist

Oilseeds Research Unit

Brady A. Vick, Research Leader
Laurence D. Charlet, Research Entomologist

Others

Claude H. Schmidt, Collaborator

In Absentia

Larry J. Heilman, Research Geneticist Insects
Theodore Shultz, Chairman, Department of Entomology, NDSU

Reviewers

Dr. Robert Faust
USDA-ARS National Program Staff
Bldg. 005, BARC-West
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Phone: (301) 344-3918

Eldean D. Gerloff
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Phone: (303) 229-5558

Dr. Don C. Zimmerman
Red River Agricultural Research Center
PO Box 5677 - NDSU Station
Fargo, ND 58105

Phone: (701) 239-1371

TRIP REPORT

Date: 8/14/90

Name: Robert M. Faust RMF

Title: National Program Leader
Molecular Entomology/Basic Insect Biology
Crop Protection

Dates of Travel: April 23-25, 1990 Location: Fargo, ND

Purpose of Travel:

Follow-up of entomology program review at Fargo, ND held August 14-16, 1989, including discussions on program restructuring and implementation of recommended actions.

Participants/Contacts:

NPS - R. M. Faust; Don C. Zimmerman, Director, Red River Valley Agricultural Research Center; S. Karl Narang, RL, Insect Genetics and Molecular Biology; Dennis Nelson, RL, Insect Biochemistry; Staff including Richard L. Roehrdanz, Robert R. Rojas, Larry J. Heilmann, Terrance S. Adams, Brad Stiles, and Maurice E. Degrugillier.

Highlights:

Discussions on new program directions within the Insect Genetics and Molecular Biology Research Unit with affected staff and the new Research Leader, Dr. S. K. Narang; Update of status of research directions since entomology review held in August 1989.

Recommendations:

A report is in preparation that will include the entomology review and the follow-up review/recommendations that had been delayed until a new RL was appointed for the Insect Genetics and Molecular Biology Research Unit. Recommended program changes have been and are being implemented via normal CRIS program adjustment actions. Emphasis in the Insect Genetics and Molecular Biology Research Unit will be shifted to Heliothis genetics, in part. Validation of the "Casanova" boll weevil male sterile strain for efficacy will be completed in the spring/summer of 1990 and transferred to Mississippi State.

cc:
E. B. Knipling
H. J. Brooks
R. R. Oltjen

8/14/90
89-NP9

Name: Brown M. Paul

Title: National Program Leader
Molecular Entomology and Insect Biology
Crop Protection

Dates of Travel: April 23-25, 1990 Location: Peoria, IL

Purpose of Travel:

Follow-up of economic impact study and meeting with ARS personnel
including discussion on program review and the reorganization of
recommended actions.

Participants/Contacts:

USDA - R. M. Foust; P. D. Frana Directors Mel Ivor Valley Agric. Center
Research Center; S. Carl Metzger, Jr., Insect Genetics and Biology;
Donald Nelson, Jr., Insect Biochemistry Staff including Mildred Hazard
Robert R. Rotan, Entomology Laboratory Supervisor S Wayne, Fred Pitts, and
Maurice S. Degenhardt.

Discussions on new program directions within the Insect Genetics and Biology
Research Research Unit with effected staff and Dir. Wayne Leander.
Dr. S. E. Moran; Update of status on research [illegible] entomology
review held in August 1989.

Recommended Use:

A report is in preparation that will include the economic results and the
follow-up review/recommendations, as that had been delayed until [illegible]
publication in the Insect Genetics Biology Research Unit.
Recommendations have been and are being implemented via normal
[illegible] and [illegible] to the Insect Genetics and
Biology Research Unit will be either to Beltsville Area Genetics, Ft
Collins, Peoria, Gainesville or [illegible] and transferred to

United States Department of Agriculture
Agricultural Research Service
National Program Staff
Beltsville, Maryland 20705

July 17, 1989

SUBJECT: Entomology Program Review at Fargo

TO: George Gassner
Research Leader
Insect Molecular Genetics and Cryobiology

FROM: Robert M. Faust RMFaust
National Program Leader
Molecular Entomology/Basic Insect Biology
Crop Protection

Following earlier discussions with you and Don Zimmerman, I have scheduled a review of the entomology research programs during my site visit to Fargo for August 14-16, 1989. The review will not include the CWUs addressing primarily screwworm research since a review (and report) was carried out earlier by Dr. Bram. I would, however, like to visit with these scientists sometime during my stay. Units involved and associated with the CWUs to be reviewed are the Insect Molecular Genetics and Cryobiology, Insect Biochemistry, and Oilseeds Research.

I am requesting your assistance in organizing and coordinating this review. As per our conversation of Thursday, July 13, 1989, a list of participants and CWUs involved was formulated and a tentative agenda agreed upon. If you have any questions concerning additional details of the review process that we may not have covered in our conversations, please do not hesitate to contact me.

cc:
T. Army
R. Bram
J. Coppedge
W. Klassen
E. Knipling
P. Miller
D. Nelson
M. Ouye
R. Soper
D. Zimmerman

7/20/89
006-NPS

Agricultural Research Service

Posted: Thu, Jul 13, 1989 5:11 PM EDT Msg: AGIJ-2834-5089
From: GGASSNER
To: DRNELSON
CC: LC.FARGO, AD.NPA, RFAUST, GGASSNER
Subj: ENTOMOLOGY PROJECT REVIEW

July 13, 1989

Subject: Entomology Project Review

To: Location, Entomologist: CWU#'s 22000 005
21350 001
24000 015
24000 003
24000 006
24000 005
24000 007
24000 002
24000 004

From: Dr. George Gassner
Review Coordinator

NPS has requested that the subject projects be reviewed during August 14-16. This review will take the form of a brief overview by lead scientists during the morning of 14 August followed. The reviewers will then visit with individual scientists at poster displays outside their laboratories or offices for the remainder of the time. All posters are to be in place from the morning of 14 August through the afternoon of 16 August. Dr. Robert Faust, NPL will lead the review and file a final report. An overview of the report will be given to the entomology scientists on the afternoon of 16 August by Dr. Faust. Should you have any questions please meet with me at 2:00 PM, Friday 14 July in the large conference room at the BRL. A review agenda will be posted by Thursday, 20 July.

cc: AD
CD
NPS

Posted: Fri, Jul 28, 1989 2:36 PM EDT Msg: BGIJ-2841-6798
From: GGASSNER
To: AD.NPA
CC: LC.FARGO, RFAUST, GGASSNER
Subj: ENTOMOLOGY PROGRAM REVIEW, FARGO

July 28, 1989

SUBJECT: Entomology Program Review, Fargo

TO: Thomas J. Army, AD, NPA

FROM: George Gassner, RL, IGCB
Program Review Coordinator

The subject review will take place on 14 - 16 August 1989, starting at 0800 hours 14 August and ending at 1630 hours on 16 August. We look forward to the presence and participation by you or your representative. An agenda and/or program background manual will follow.

cc: Faust
Klussen
Zimmerman

Posted: Mon, Aug 7, 1989 9:43 AM EDT Msg: IGIJ-2845-7805
From: RFAUST
To: GGASSNER
CC: AD.NPA, DZIMMERMAN, RFAUST
Subj: Future Res. Program

August 7, 1989

SUBJECT: Future Research Program of the Insect Molecular Genetics and Cryobiology Laboratory

TO: George Gassner
Research Leader, IMGCL

Dennis Nelson
Research leader, IBL

FROM: Robert M. Faust RMF
National Program Leader
Molecular Entomology/Basic Insect Biology
Crop Protection

Prior to our scheduled meeting of August 14, 1989, please furnish me with the following information:

1. Concise descriptions from your perspective as Research Leader of the future research program and thrusts that you feel should be undertaken in the Insect Molecular Genetics and Cryobiology Laboratory. Please be specific in the descriptions of your proposed thrusts. In developing this material please indicate the personnel that will be associated with each project and also include, in addition to the problems to be addressed and the specific scientific and technological objectives for each proposed project, a brief statement of the approaches (include target insects, commodity, etc. when appropriate). Also, please keep in mind the resources available to you, that the research projects are responsive and address national problems, issues, and priorities, fall within the mission of the Agency, and are consistent with the ARS Program Plan. The current ARS Program Plan (6-Year Implementation Plan, 1986-1992) is suggested as a guide and reference source for the ARS high-priority National Programs. My understanding is that you have already been undertaking discussions with Don Zimmerman aimed at this activity.

   Construct the format in such a way that it will allow us to use the material as a working document in our forward planning discussions/project development. This document will also serve as a "blueprint" as we finalize decisions on program direction and provide additional guidelines for the research objectives, funding, SY allocation, research priorities, and resource needs.

2. A 1-page summary that describes the major contributions of the Laboratory's overall program in advancing science and technology from the perspective of the scientists (I leave the time frame to be covered at the scientist's discretion). Please use the following format.

8/7/89
043-NPG

Major Contributions	Impact on Science	Impact on Technology
1.		
2.		
3.		
etc.		

I hope that you will be able to respond to this request so that I may become familiar with it prior to our final discussions near the end of the review process. In any event do the best you can and we will go with whatever you can put together.

cc:
W. Klassen
T. Army
D. Zimmerman
R. Soper
J. Coppedge

Posted: Tue Mar 14, 1989 8:16 PM EST Msg: NGIJ-2769-8060
From: LC.FARGO
To: ADA.PNR.NPS
CC: ad.npa, rfaust, rbram, jr.coppedge, rsoper, pamiller
Subj: Fargo Entomology Realignment

This is a follow-up to my Telemail message of March 9, 1989. The two entomology units will be divided as indicated in the previous memo, the only change being the name of Dr. Gassner's new unit. He prefers the name "Insect Genetics and Cellular Biology." I approve this name.

I had earlier considered the possibility of moving Dr. John Barker to the Oilseeds Research Unit but after discussions with Dr. Nelson and Dr. Vick, I have decided to leave him in the Insect Biochemistry Unit. The final realignment of the two units is the same as in my March 9 message and is repeated below.

INSECT GENETICS AND CELLULAR BIOLOGY

Dr. George Gassner, Research Leader
Dr. Maurice E. Degrugillier, Research Entomologist
Dr. Larry Heilmann, Research Geneticist
Dr. Richard Roehrdanz, Research Geneticist
Mr. David T. North, Research Geneticist
Dr. Ian C. McDonald, Research Geneticist
Dr. Roger A. Leopold, Research Entomologist
Dr. Robert Rojas, Research Biologist
Dr. Brad Stiles, Research Biologist

INSECT BIOCHEMISTRY

Dr. Dennis R. Nelson, Research Leader
Mr. J. George Pomonis, Research Chemist
Dr. Terrance S. Adams, Research Entomologist
Dr. John G. Riemann, Research Entomologist
Dr. John Barker, Research Entomologist
Dr. David Taylor, Research Entomologist
Dr. Leslie Hammack, Research Entomologist
Dr. Richard D. Peterson, Research Entomologist

I would like to request formal approval of this realignment effective immediately. The division of existing funds and development of CRIS units will be completed by the beginning of the next fiscal year.

Don C. Zimmerman, Director

cc: T. Army, AD
R. Faust, NPS
R. Bram, NPS

cc: T. Army, MD
R. Rust, Jr.

APPENDIX B. Workshop Recommendations for ARS Research on Molecular Entomology and Insect Genetics, ARS National Leaders' Meeting, April 10-13, 1990, Washington, D.C.

Workshop No. 22: Molecular Entomology and Insect Genetics: Program Development

Discussion Leader: Robert M. Faust

Objective:

Provide a foundation for the development of near-term program strengthening, coordination and implementation actions for insect genetics and contemporary molecular entomology as components of ARS research on alternative insect control strategies and enhancement of beneficial insects.

Research and Technology Needs:

- Research is needed on endogenous bioregulators governing insect development and regulation in order to further an understanding of the genetic basis for biological functions in biochemical/molecular terms.

- There is a need for research to identify, isolate, and map important genes of the insect genome.

- Research is needed to develop technology and vector systems to efficiently transfer and incorporate manipulated genes into insect host genomes.

- Research is needed to elucidate the underlying molecular mechanisms that determine the efficacy of predators and parasitoids and to enhance the effectiveness of these agents and other beneficial insects.

- There is a continuing need for well-focused research efforts related to genetic manipulation of entomopathogens to enhance their effectiveness as biocontrol agents.

- There is a need for the development of creative technology to engineer host plants, entomopathogens, and other non-pathogenic associated microorganisms, such as commensals, as novel vector-delivery systems of agents that disrupt insect life processes.

- Research is needed on gene engineering of host plants to confuse the host-finding sensors of pest insects.

- The genetic basis of back-cross sterility in Heliothis virescens needs to be better understood so that it can be extended to other Heliothis sp. such as H. zea.

- Research is needed on the molecular basis for immune responses of vertebrates to insect pests and in the development of immunologically-based technologies for management of populations of blood-feeding arthropods that affect man and animals.

(Workshop No. 22)

- Research is needed on the use of DNA technology to improve our understanding of the biosystematics and population genetics of economically important insects.

- Focus of molecular entomology research should be aimed at selected major pests that are currently or potentially targets of suppression systems. Potential model systems for use in molecular entomology might include, but are not necessarily limited to, Heliothis sp., tephridid fruit flies, honey bee, mosquitoes, and boll weevil.

Recommended Actions:

The overriding recommendation centers on the development of a strategic plan for organizing ARS molecular entomology research. Recommended actions that will be covered by the plan include --

- Develop an inventory of the current ARS molecular entomology research program and a description of its relationship with other research.

- Develop a descriptive listing of major global issues on which molecular entomology could have an impact.

- Establish the research and technology needs, objectives, thrusts, focus areas, and research approaches for the ARS molecular entomology program (this workshop, in part).

- Establish an organizational structure to meet the needs of the program and to facilitate networking, clientele visibility, linkages and coordination, and cooperative relationships.

- Develop an implementation plan of action for the strategic plan.

- Define program constraints and constraints that could hinder implementation.

- Define the resources needed to fully implement the proposed strategic plan.

- Research is needed on the use of GIS technology to improve understanding of the biology, politics and implementation results of economically important laws.

- Focus of molecular entomology research should be aimed at selected major pests that are currently or potentially target on implementation system. Potential pests for use in molecular entomology might include, but are not necessarily limited to, Helicoverpa zea, Lepidoptera fruit flies, honey bee, mosquitoes, and boll weevil.

Recommended Actions:

The overall APHIS recommendation centers on the development of a strategic plan for implementing APHIS molecular entomology research. Research and action that will be covered by the plan include --

- Develop an inventory of the current APHIS molecular entomology research programs and a description of its relationship with other

- Develop a descriptive listing of any kind leaves an white molecular entomology could have in impact.

- Establish the research and technology needs, objectives, phases, future areas, and research approaches for the APHIS molecular entomology program (this workshop, in part).

- Establish an organizational structure to meet the needs of the program and to facilitate cooperation, citizenship visibility, leadership and coordination, and cooperative relationships.

- Develop an implementation plan of action for the strategic plan.

- Define programs constraints and constraints that could hinder implementation.

- Define resources needed to fully implement the proposed

APPENDIX C. SCA 58-82HW-8-7 on Genetic Sexing, Major Accomplishments

Major Accomplishments for SCA 58-82HW-8-7 during FY 86-88

* Characterized two genes in Boll Weevils each of which the expression is unique to one sex.

1. Vitellogenin gene of females
   a. Can be used to mass rear males only
   b. Useful in studies of hormonal control of female fertility in boll weevils

2. Protamine gene of male
   a. First time isolated from an arthropod
   b. Required for the production of sperm
   c. Could be used to sterilize males (will not use chemicals or ionizing radiation)
   d. Can be used to mass rear males only
   e. Has potential use for ablation of specific organs and tissues for studies of physiology and development.
   f. Patent applied for

3. These and other gene probes will be useful for -
   a. Markers in mass release experiments
   b. Assessing genetic variability in wild populations

* Characterization of prepromelittin gene and production of genomic DNA library for honey bees at Marquette University.

* Determined that Drosophila transposable elements were not useful for transforming other insect genomes.

a. This accomplishment would have taken 4 to 6 years for several ARS scientists working in a less focused manner.
b. A highly effective screening system for rapid detection of transformed systems was developed.

* Characterization of vitellogenin genes and chorion genes of the mediterranean fruit fly by Harvard and Crete Universities.

* ARS scientists at five locations were given an opportunity to work with other molecular biology teams in laboratories in Wisconsin, Cambridge & Crete.

a. At least 4 ARS scientists participated.
b. A Fargo ARS scientist spent 18 months at Marquette University training the senior scientist and post doctoral teams in cutting edge NIH techniques.
c. This focused effort at Marquette University resulted in the above boll weevil gene discoveries and boll weevil gene libraries.

* Overall 5 post doctoral scientists trained in and contributed to the program.

* International molecular biology and biotechnology symposium by ARS and University cooperators during Joint FAO/IAEA meeting at the University of Crete, Kolymbari, Crete, Greece, Sept. 3-5, 1988.

APPENDIX D. Background, Workshop Recommendations and Summary of Results of the Sterile Boll Weevil Program

United States Department of Agriculture
Agricultural Research Service
Northern Plains Area
Red River Valley Agricultural Research Center
Biosciences Research Laboratory
P.O. Box 5674
State University Station
Fargo, North Dakota
58105

22 February 1989

SUBJECT: Starkville, MS, Boll Weevil Scientist Visit 6-8 March 1989

TO: R. Leopold*
I. McDonald*
D. North
R. Rojas
R. Roehrdanz
B. Stiles
L. Heilmann

FROM: George Gassner

Dr. J. Smith and Dr. W. McGovern will visit the BRL arriving on the afternoon of 6 March and departing on or about 11:00 AM 8 March. The visit has three objectives:

7 March
0800
Small Conference Room, BRL

1. Introduce BRL IBMG scientists working in BW research.
2. Familiarize the visiting scientists with IBMG research on BW.
3. Learn what Dr. Smith and Dr. McGovern consider as priorities for the BW control program.
4. Prepare a report to be used by NPS during the 23 March Panama City BW working group meeting to determine future BW research objectives.

8 March - Until Departure
Finalize Report

The above lead scientists (*) will arrange time for individual scientists and laboratory visits. All participants are encouraged to join together for lunch and dinner on 8 March.

The visitors will be lodged at the Fargo Raddison Hotel.

cc: D. Zimmerman
M. Nyquist
T. Army
W. Klassen
J. Coppedge
R. Faust
IBMG scientists

United States Department of Agriculture
Agricultural Research Service
National Program Staff
Beltsville, Maryland 20705

April 18, 1989

SUBJECT: Clinton, Louisiana, Strain Evaluation for Fargo Select Boll Weevil Strain

TO: Bob Faust
Waldemar Klassen

FROM: James R. Coppedge, NPL JRC
Field Crop Entomology

At 3:30 pm today, I was involved in a conference call with Drs. Putnam, Schilfgaarde, Smith, and Gassner concerning the subject test. The results of this conversation were as follows:

1. The test will be conducted if Fargo can demonstrate that their strain is healthy and competitive under laboratory conditions (by May 15).

2. The test will be conducted as last years's by Dr. Villavaso. Researchers from Fargo will visit test site 3-4 times.

3. The funding for the project will come from the Boll Weevil Lab ($4000), Fargo Lab ($6000), Mid-South Area Office ($5000), and the Northern Plains Area Office ($5000).

4. The necessary arrangements to initiate this test are to begin ASAP.

United States Department of Agriculture
Agricultural Research Service
National Program Staff
Beltsville, Maryland 20705

May 18, 1989

SUBJECT: Report of the Working Conference on Cotton Boll Weevil Research Held in Panama City, Florida, March 22-23, 1989

TO: Waldemar Klassen, ADA
Plant and Natural Resource Sciences

FROM: Robert M. Faust RMF
National Program Leader
Workshop Chair

James R. Coppedge JRC
National Program Leader
Workshop Co-Chair

Attached herewith is the final report on the ARS Boll Weevil Sterility Workshop held in Panama City, FL, March 22-23, 1989, for your information.

The evening of March 22 was devoted to informal discussions (pros and cons of the program) with an open agenda. A structure workshop was held the following day and individual brief reports detailing current research and knowledge gaps, future research activities, resources needed and projected target dates for completion, and recommendations were presented. Page 6 of the report includes an amendment (dated April 18, 1989) to the research activities.

Enclosure

cc: w/encl
E. B. Knipling

WORKING CONFERENCE
ON
COTTON BOLL WEEVIL
RESEARCH

PANAMA CITY, FLORIDA

MARCH 23, 1989

WORKING CONFERENCE

ON

COTTON BOLL WEEVIL

RESEARCH

PANAMA CITY, FLORIDA

MARCH 22, 1969

ARS Boll Weevil Sterility Program
"Past, Present, and Future Perspectives"

Panama City, Florida

March 22 & 23, 1989

Attendees

Name	Affiliation (Lab, Location, Phone)
R. M. Faust	USDA-ARS-NPS, Bldg. 005, BARC-W, Beltsville, MD 20705 - 301-344-3918
Eric Villavaso	USDA-ARS, Boll Weevil Research Unit, Box 5367, Mississippi State, MS - FTS 497-1190
Bill McGovern	USDA-ARS, Boll Weevil Research Unit, Box 5367, Mississippi State, MS - FTS 497-1180
Jim Smith	USDA-ARS, Boll Weevil Research Unit, Box 5367, Mississippi State, MS - FTS 497-1133
Dick Hardee	USDA-ARS, SFCIML, Box 346, Stoneville, MS 38776 FTS 497-2231
James Coppedge	USDA-ARS-NPS, Room 114, Bldg. 005, BARC-W Beltsville, MD 20705 - FTS 344-1541
T. B. Davich	USDA-ARS, Boll Weevil Research Unit, Box 5367, Mississippi State, MS 39762 - FTS 497-1102
David T. North	USDA-ARS, Biosciences Research Lab, Box 5674, Fargo, ND 58102 - 701-239-1288
Andy Terranova	USDA-ARS, Cotton Production Research Unit, Box 2131, Florence, SC 29503 - 803-669-6664
Jimmy Pendergrass	National Cotton Council, P. O. Box 12285, Memphis, TN 38182 - 901-274-9030
Bill Grefenstette	USDA-APHIS-PPD, Room 814, 6506 Belcrest Rd., Hyattsville, MD 20782
Joe Leggett	USDA-ARS, Western Cotton Research Lab, 4135 E. Broadway, Phoenix, AZ 85040 - FTS 261-3524
Don C. Zimmerman	USDA-ARS-PRVARC, Box 5677, Fargo, ND 58105
George Gassner	USDA-ARS-NPA, Biosciences Research Lab, Box 5674, Fargo, ND 58105 FTS 239-1270

Panama City, Florida

March 22 & 23, 1989

EXECUTIVE SUMMARY

The boll weevil is recognized as the most important single agricultural insect pest in the United States. (See Attachment 1)

An eradications effort by APHIS is now underway in the eastern part of the cotton belt and scheduled to cover this entire cotton belt within the next several years.

A sterile male technique has been developed by ARS as a suppression component for use in eradication. (See Attachment 2)

Sterile male technology has not been adopted by APHIS because:

It has not been conclusively proven in large-scale testing (Attachment 2).

It is generally believed that a high ratio of sterilized to wild weevils must be maintained.

Sterile male technique was not designed to eliminate heavy infestations.

The reasons the program needs the technology follow:

It would be of great value in areas that are environmentally sensitive because it is species specific and has no direct effect on other environmental components.

Its greatest value would be against boll weevil populations along the northern limits of the boll weevil' range and in buffer areas between eradicated and uneradicated areas.

Eradication of the boll weevil would make management of the remaining pests easier, less costly and economically more satisfactory.

EXECUTIVE SUMMARY

Proposed Immediate Research Directions

The working group on sterile boll weevils proposed the following:

Initiate a selection program for post irradiation survival and mating propensity in the strain of boll weevils presently being reared at the Gast Boll Weevil rearing facility and a previously selected strain starting April 1989.

Continue competitive test in isolated cotton at Louisiana Agricultural Experiment Station, Clinton, LA during summer of 1989.

Continue to improve effectiveness and efficiency of producing sterile boll weevils.

Carry out a competitive test on the Gast Laboratory reared strain after six generations of selection during summer 1990.

Plan and carry out a field trial in a suitable area during the summer of 1992.

Proposed Continuation of Long-Term Molecular Biology Research

Ongoing long-term research should continue on genetically engineered all male strain, sterility in the absence of ionizing radiation, and biosystematics. (Attachments 3 & 4).

This research would eliminate the debilitating effects of chemicals and ionizing radiation.

(1) Additionl Activities Needed

(2) Dates

(3) Resources

1. Mass Rearing

   (a) Mass Rear Select (Fargo) Strain

   (b) Select Starkville Strain - 6 Generation

   (c) Starkville Strain - No Selection

      (1) TBA (Gast)

      (2) TBA Fargo 3 Gen/Gast 3 Gen/Fargo 3 Gen

      (3) Present Resources (Gast)

2. Retest Select Strain - Small Plot Field Test - Clinton, Louisiana - $20,000 April - June 1989

3. Field Trapping, Population Dynamics of Weevils in Isolated Test Areas.

4. Field Test Mass Reared Starkville Strain

   Size - ca. 1500 Acres

   Date - 1990

   Cost - $50,000

   Detail Operational Plan in Place by March 1990.

5. Field Test Selected Starkville Strain - 1991

6. Decision Time - 1992

   A. Starkville Select

   B. Fargo (Cassanova) Strain

   C. Future of Sterile Boll Weevil

Attachment 1

Under the sterile insect technique, boll weevils are reared in the insect production facility and treated with sterilizing radiation and chemical sterilizing agents. The sterilized adults (both males and females) are then released within infested fields. The sterile weevils mate with wild (fertile) adults, resulting in the production of sterile (nonviable) eggs. Sterile weevils are released at a rate of 300-400 adults per acre per week over a 10-week period extending from early June through mid-August. The initial application is made prior to the pinhead square stage of cotton development, and a large population of sterile weevils is maintained in the field throughout the period of natural boll weevil reproduction. Weekly monitoring, sorting, and identification of trapped weevils are necessary to ensure that a sufficiently large sterile population is maintained within the treatment area. Field studies conducted during 1987 and 1988 indicate that the sterile insect technique is an effective suppression tool in areas where weevil populations are low, and during early to mid-season periods before peak populations occur.

United States Department of Agriculture
Agricultural Research Service
Northern Plains Area
Red River Valley Agricultural Research Center
Biosciences Research Laboratory
P.O. Box 5674
State University Station
Fargo, North Dakota
58105

October 16,1988

SUBJECT: Performance Appraisal of Fargo Select Strain
TO: Frank Enfield
Willian McGovern
James Smith
Eric Villavasso
FROM David North

Attached is a copy of the one page summary of the performance appraisal of the Fargo selected strain that I gave Waldy Klassen while he was here last Thursday.:

We spoke briefly regarding the continuance of field tests at Clinton,La.or a similar location;the possible adaptation of the selected strain to mass rearing;and the need for an informational meeting with ARS people in Beltsville regarding future research and funding and then an information meeting with a group of interested persons at the Beltwide Cotton Conference in Nashville in early January. He was receptive to all of this and thus we can proceed.

cc: G. Gassner
I. McDonald

Performance Appraisal of Fargo Select Boll Weevil Strain vs. Other Strains

David T. North
Res. Geneticist
USDA,Fargo,ND

Frank Enfield
Prof.,Genetics
Univ. Minnesota

Eric Villavasso
Res. Ent.
USDA, Miss. State

Characteristic	Select	Starkville	Ebony	Comp
		STRAIN		
14 Day Post-Irrad... Survival(10Krad)	95%	30%	35%	----
10 Day post-Irrad... mating propensity	70%	15%	25%	
9-10 Day post-Irrad... frass production mg .	1.07	---	0.46	0.74
LT 50 (10 Krad) Field/Lab	21.7/16.2	15.8/11.3	----	16.6/11.8
		Attractiveness 2 Males per Trap (5 reps)		
A. Days 2-6	175	217	----	123
B. Days 7-11	193	147	----	130
C. Days 12-16	145	26	----	26
% Males that mate and transfer sperm 3 consecutive days post-irradiation (10Krad)	93.3%	38.0%		
Competitiveness of released irrad. males in field plots after 14 days	25.0%	25.0%	*(Clinton,La. 1988)*	

Attachment 2

Research on boll weevil sterility was begun in the mid 1960's. Both irradiation and chemosterilization were considered, but each was found to be lacking in one or more areas of critical need. Early research set high standards of performance for sterile weevils. Early tests were unable to measure up to those standards. The few field tests that were conducted were often of too large a scale to be properly evaluated, and they were conducted without controls. In the late 1970's realistic standards of performance for sterile weevils were established. Sterile males must be able to attract and inseminate native females for 7 days after release. Recent advances in mass-rearing technology and strain selection have resulted in the production of vigorous, disease-free weevils that were better able to withstand the ill effects of irradiation. Additionally, methods of preventing residual fertility in irradiated females without reducing their vigor or that of the sterile males have been developed so that there is no need to separate the sexes before release. Now weevils are dipped in an aqueous solution of diflubenzuron prior to irradiation , this produces weevils with higher levels of competitiveness and sterility that approaches 100%.

Small plot tests (1/8 - 1/2 acre) that provided measurements of the relative competitiveness of sterile and fertile weevils were developed in the late 1970's. This technique provided a means of determining whether changes in sterilization methods that looked promising in the laboratory could be expected to produce better results in the field as well.

In 1983 and 1984 the first controlled tests of the effectiveness of sterile weevils against natives on commercially grown cotton were conducted in both the delta and hill counties of Mississippi. In 1983 egg hatch of native females was reduced from 98% in control fields to 53% in fields receiving sterile weevils. In 1984, a year with a much lower than normal population of native weevils, hatch was reduced from 94% in control fields to 15% in fields receiving the sterile weevils. In both years, the tests were conducted on between 100 and 180 acres of commercially grown cotton.

In 1987 and 1988 a large scale test encompassing 3-5000 acres was conducted in west-central Alabama. Weekly releases of sterile weevils proved their effectiveness against native populations particularly in fields that were greater than 10 acres in size. The test also confirmed the ability of the USDA-ARS mass rearing facility to consistently produce large quantities of vigorous mass-produced and sterilized boll weevils.

Genetic selection of a strain of weevis in which irradiated males have an extended lifespan and an increased propensity to inseminate females was begun about 1980. Research was a joint venture between scientists at the USDA-ARS Bioscience Research Laboratory, Fargo, ND, and the University of Minnesota. The extended post-irradiation life and mating propensity of the selected males was duplicated at Mississippi State, MS. several replications of males reared and sterilized at the Fargo laboratory were utilized in the study.

Future research on boll weevil sterility should be directed toward improving the efficiency of procedures utilized to mass produce and sterilize boll

weevils, measuring the field competitiveness of the genetically selected strain as well as incorporating additional desirable qualities into the strain, determining the effectiveness of the selected strain after is has been subjected to mass rearing for several generations, and defining the situations in which the use of sterile weevils could be of maximum benefit to the boll weevil eradication program.

Attachment 3

With the goal of using molecular biological techniques to develop a genetic sexing technique and possibly eventual genetic control techniques for the boll weevil *Anthonomus grandis* work on the molecular biology of this insect was initiated in January 1987. After initial construction of a series of genomic and cDNA recombinant libraries the project has gone along three main routes. The common objective is to obtain some insight into the molecular genetics of *A. grandis* and to isolate various genes and gene promoter regions that could prove useful in future genetic engineering experiments with this insect. Emphasis has been placed on sex-specific gene expression with potential use in genetic sexing for production of single sex or male sterile lines.

I. Using cloned probes isolated from other organisms, particularly *Drosophila melanogaster*, genomic souther blots of A. grandis were hybridized at several levels of stringency. This was done in hopes that there might be enough homology that cross-hybridization could be used to isolate the corresponding *A. grandis* genes. More than twenty different genes have been analyzed in this way. Most gave negative results including the chorion and yolk protein genes, the sex control genes, and a series of *Drosophila* transposable elements. Positive hybridization results were obtained with actin, amylase, heat shock and histone probes. Two different actin genomic clones have been purified. Although this method has not yet been successful in isolating sex-specific genes we will continue to screen potential clones as they become available.

II. Messenger RNA was extracted from male and female weevils and cDNA recombinant libraries constructed from each in the vectors lambda gt10 and gt11. Each library was in turn screened with a labeled cDNA from each of the male and female mRNAs. This cross-screening yielded a number of clones that were either male or female specific. These have now been extensively characterized and sequenced. Genomic clones have been isolated and are being characterized.

A male-specific cDNA was sequenced and found to be 743 bp in length. An open reading frame of 132 amino acids was found to code for a very arginine rich protein with similarity to protamines, a sperm specific DNA binding protein previously described only in vertebrates. Northern blots of RNA extracted from different tissues and from different developmental stages showed that this messenger was found only in the testes and only in the adult or late stage pupae. Genomic southern blots showed that this sequence was a member of a small gene family of 4-6 copies. Three different genomic clones have now been isolated from a genomic library constructed in the vector EMBL3. These genomic clones are now being mapped and characterized. We hope to map and sequence the male specific promoter regions in the near future.

A female-specific cDNA of 1223 bp was purified and sequenced. An open reading frame was found but the amino acid sequence was not similar to any other sequence in the protein sequence data banks. Northern blots showed the sequence was complementary to a very large 6.5 kb female specific mRNA. We

now know that this sequence is a portion of the *A. grandis* vitellogenin cDNA from a different portion of the gene than the cDNA isolated by antibody methods (see next section).

III. To obtain clones of the female-specific vitellogenin gene an antibody screening procedure was used. *A. grandis* has two very abundant proteins in its eggs. The most abundant is a very large 160 kilodalton protein and the other is about 47 kilodaltons. The large protein was purified on a polyacrylamide gel and antibodies to it prepared by injection into rabbits. These antibodies were used to screen a lambda gt11 expression library made from female *A. grandis* RNA. A 661 bp clone was purified and sequenced. The entire sequence consisted of a single open reading frame indicating that this was an internal fragment of a larger message. It was homologous to a large 6.5 kb message which was female-specific. This clone was used to screen a genomic library and two overlapping clones were isolated. Southern blots had shown that this was a single copy sequence. The female-specific cDNA previously found also hybridized to the same genomic clones although in different positions. We therefore have two pieces of a large cDNA and two genomic clones containing the gene for this protein. We are now mapping the transcripts from this gene and subcloning portions of it. The goal is to isolate the sequences controlling the female-specific expression of this gene.

FUTURE WORK

We have isolated and sequenced some male and female-specific cDNA clones from *A. grandis*. The male-specific clone codes for a testes specific protamine, the first protamine found in invertebrates. If the promoter regions of this gene can be isolated they have potential use in creating sterile male lines. The promoter sequences can be linked to a toxin gene or any other deleterious gene and reinserted into the boll weevil genome. The new sequence would be expressed only in the adult testes, destroying that organ and rendering the insect sterile. The female-specific yolk protein promoters could be used in a similar manner to kill all the females in a line, leaving only males for sterilization and mass release. We have the clones isolated and are in the process of mapping and isolating the promoter sequences that control them.

The next major hurdle to genetic engineering in boll weevils or any other insect is finding a method to genetically transform them with the purified gene of interest. The P-element system of *Drosophila* works only in very closely related species and will probably not be useful. It will be necessary to find another way. The best way will probably be to isolate and use transposable elements endogenous to the organism of interest. This means that transposable elements will have to be isolated from boll weevils.

With the clones already isolated and with others from the libraries it will be possible to do this by screening DNA from many different lab strains and geographic isolates searching for restriction fragment length polymorphisms (RFLPs). Some of these will be due to the insertion of genetic elements that have transposed into the sequence being analyzed. This method will also have the added benefit that the RFLPs found will be very useful for

Attachment 4

The mitochondrial DNA (mtDNA) of animals has proven to be an extremely powerful tool for studying systematics and evolution of mtDNA is normally a circular DNA molecule of 15-20 kilobases (kb). The small size and sequesterization outside the nucleus in the mitochondria make it possible to differentially separate it from nuclear DNA. The molecule seems to evolve rapidly with respect to its primary nucleotide sequence perhaps 5 times faster than comparable nuclear DNA. As a result mtDNA often gives rise to extensive sequence heterogeneity among individuals of the same species. MtDNA is maternally inherited and the variation present in natural populations can be used to reconstruct maternal lineages and phylogenies both within species and among closely related species.

The restriction fragment patterns of mtDNA from the cotton boll weevil, Anthonomus grandis Ebony strain, using 14 restriction endonucleases were evaluated. The cleavage sites for 12 of the enzymes have been mapped, i.e. their location and relative order on the DNA molecule has been determined. Restriction fragment pattern polymorphisms have been used to demonstrate that differences can be detected among weevil populations of diverse origin (Ebony strain, "Thurberia" weevil from Arizona, Mexican weevil collected on Hampea nutricia in southern Mexico and a cotton weevil from El Salvador). This technique has a high potential for the study of biosystematics, evolution and dispersal of the boll weevil along with assessments of the genetic variability in natural populations of the weevil.

FIELD TESTING OF A RADIATION RESISTANT STRAIN OF THE BOLL WEEVIL

E. J. Villavaso, W. L. McGovern, D. T. North & J. W. Smith
USDA, ARS, Boll Weevil Research Unit, Mississippi State, MS

About ten years ago, a strain of boll weevil that exhibited extended postirradiation survival in the laboratory (RR) was developed through a research agreement between the USDA Metabolism and Radiation Research Laboratory, Fargo, ND (MRRL) and the University of Minnesota. The strain from which the RR strain was developed (CP) came from a broad base laboratory strain that had been synthesized from 6 strains collected from College Station, TX, Florence, S. C., Monroe, LA, Tifton, GA, Clay County, AR and Selma, AL and a long established laboratory strain from Mississippi that was homozygous for ebony body color. Cooperative research between MRRL and the Boll Weevil Research Unit, Mississippi State, MS to evaluate postirradiation survival, attractiveness, mating ability and competitiveness in field, field cage, greenhouse and laboratory environments was begun in 1982 and continued in 1987, 1988 and 1990.

In 1982 the RR and CP strains and the strain long under mass production at Mississippi State, MS (ST) were reared and irradiated in Mississippi (-MS) and tested for competitiveness in isolated 1/4 acre field plots and for longevity in the laboratory. Two groups of Starkville males were tested. One was mass-reared (ST-MR) and the second was reared on a small scale along with males from the RR and CP strains. LT_{50}'s averaged 12.0, 11.7, 8.7 and 7.0 days for the RR-MS, CP-MS, ST-MS and ST-MR groups, respectively ($12.0=11.7>8.7=7.0$; $P < 0.01$). Both of the North Dakota strains lived significantly longer than both Starkville groups, but the expected 21 vs 13 day difference in longevity between the RR-MS and CP-MS groups was not observed. Differences in competitiveness

over the 7 day period of the test were not significant. Competitiveness averaged 44% which was identical to a similar test conducted with the ST-MS strain the previous year.

In 1987 RR, CP and ST males were reared and irradiated in North Dakota (-ND) and shipped to Mississippi where postirradiation survival, attractiveness and mating ability were measured on males that were held in 1" screened plastic boxes on cotton plants in the greenhouse or screened field cages (12'X24'X6'). LT_{50}'s averaged 19.5, 15.7 and 15.2 days, respectively, for RR-ND, CP-ND and ST-ND males ($19.5>15.7=15.2$; $P < 0.01$ RR-ND males were able to inseminate more females 14 days after irradiation and were more attractive than either CP-ND or ST-ND males during the second week after irradiation, but neither of these differences were significant. Had a field test been conducted in 1987, the longer-lived, more attractive, higher mating propensity RR-ND males would have been expected to be more competitive during the second week after release.

In 1988 RR and CP males, reared and irradiated in North Dakota, were tested for competitiveness during the second week after release and for postirradiation survival in isolated 1/4 acre plots of cotton. Competitiveness for days 8-14 after release averaged 24% for both groups. LT_{50}'s were not significantly different and averaged 17.5 and 16.5 days for the RR-ND and CP-ND strains, respectively. This difference was not as great as the 3.8 day difference seen in 1987. Thus, the expected greater competitiveness during the second week after release did not occur.

In 1990 RR and ST males were again tested for competitiveness and postirradiation survival in 1/4 acre isolated field plots. Males of the

RR and ST strains reared and irradiated in North Dakota and males of the ST strain mass-reared in Mississippi were tested for competitiveness and postirradiation survival. LT_{50}'s were not significantly different and averaged 14.8, 13.8 and 14.0 days for the RR-ND, ST-ND and ST-MR groups, respectively. In 1990, unlike in previous years, virgin females were not released only on the first day of the test as were the males, but were also released on the 3rd, 7th & 10th days. Instead of evaluating competitiveness on only the 7th or 14th days after release, competitiveness was evaluated on the 3rd, 7th, 10th and 14th days after release. Treated males were unfed and 3 days old at the time of release and control males were 5 days old and diet fed at the time of release. This is the first time such a combination of treated and untreated weevils have been tested, and hindsight tells us that the test was probably significantly biased in favor of the untreated males. Competitiveness ranged from a low of 4% (RR-ND; days 0-3) to a high of 38% (ST-MR; days 7-10) over the course of the test. Differences in relative competitiveness of the strains were not significant.

The low overall competitiveness seen over the course of the 1990 test apparently occurred because the young, unfed treated males were not able to produce as much pheromone or sperm as the older control males during the first 3 days after release. This is the period when most of the mating probably took place. The number of released weevils remaining in the field diminished significantly between third and seventh days after release and continued to diminish through the end of the 14 day test probably as a result of predation by fireants. We don't know if this would occur in commercially grown cotton that is frequently subjected to insecticide treatments.

Eric Villavaso

APPENDIX E. Communications Relative to Genetic Sterility in *Heliothis*

United States Department of Agriculture
Agricultural Research Service
Northern Plains Area
Northern Plains Soil and Water Research Laboratory
1500 North Central
P. O. Box 1109
Sidney, Montana 59270

October 22, 1989

Subject: Sterility in *Heliothis [Helicoverpa] zea*.

To: Dr. Dick D. Hardee, Laboratory Director,
Southern Field Crop Insect Management Lab
USDA/ARS P.O. Box 346
Stoneville, MS 38776

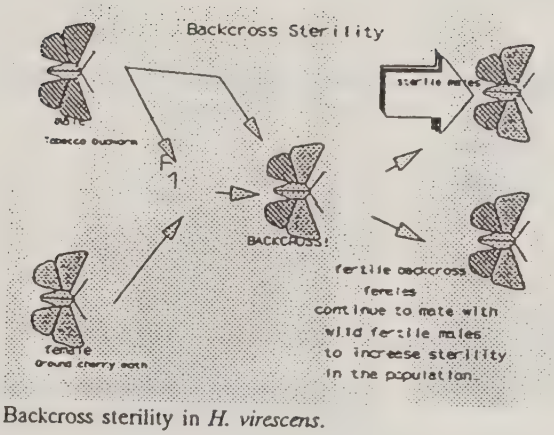
From: Neal R. Spencer, Research Entomologist
Biological Control of Weeds Research Unit
USDA/ARS P. O. Box 1109
Sidney, Montana 59270

While working on the backcross sterility of *H. zea* in the spring of 1988 in your lab, we successfully crossed a male *H. assulta* (SEE ENCLOSED TABLE) with a female *H. zea* from our stock colony. The records of this work are in the quarantine facility and additional information has been given to Dr. Laster. One cross produced only males. At the time this work was going on, I sent reproductive tissue to Dr. Maurice DeGrugillier at the Fargo lab for analysis. He identified virus-like particles in the testes of the preserved material. This was discussed with Dr. King at that time. I felt that we had found a pathogen that could be used for *H. zea* control, *if* we could rear the cross. That was a large *if* since we lost 100 % of our stock (FROM THE PATHOGEN?). I did not get Dr. King interested in following this line of research and since I was in the process of transferring to Montana, I was unable to continue the work.

The cross between *H. assulta* ♂♂ and a *H. zea* ♀♀ transferred at least one and perhaps three different insect viruses. One of these may be the sterility factor needed for *H. zea* control.

I discussed this research with Maurice this past week. He is of the opinion that another insect virus is responsible for the sterility in *H. virescens*. The virus is transmitted by *H. subflexa* into the *H. virescens* line and after several generation of backcrosses, all males carry the sterility factor.

Maurice believes that the males carry a virus which attacks the mitochondria in the testes.



Backcross sterility in *H. virescens*.

Dr. Robert M. Faust was a participant at a review of the Fargo Lab a month ago. He feels that this area of research is worthwhile for the ARS mission and goals. Dr. E. M. Dougherty feels that it would not be difficult to implant viral DNA into a *Heliothis* species egg. If this is true, perhaps the virus from *H. virescens* could be transferred to *H. zea* using one of the new biotechnology methods. This week, Maurice Degrugillier is at a Molecular Insect Science Meeting in Phoenix. I believe that it would be best that you discuss this research with Maurice this next week, if you are interested. He is a pleasant person and has conducted research along these lines for some years.

In my estimation, this line of research could be as significant as that of the original work on the backcross sterility of *H. virescens*. This proposed research would take time, money, effort and the cooperation of several labs. Technology for the genetic control of *H. zea* may be the end result.

The other missing link is that of *H. helenae* from the island of St. Helena. This species may be closer still to *H. zea* and contain a sterility factor.

Dr. Hardee, I am out of the *Heliothis* research and back into the biological control of weeds. I am interested in the development of *H. zea* sterility since I spent some time in that area. I hope that I have been helpful and have contributed to a problem's solution. Good luck in your future endeavors.

cc:

Dr. Maurice DeGrugillier
Dr. J. Powell
Dr. P. C. Quimby
Dr. Eldean Gerloff

H. ZEA females (Stoneville) X H. ASSULTA males (Pakistan)	*Total number*	pupal weight in mg.
larvae collected	*431*	
larvae dead (2/9/88)	*120*	
larvae pupated (N. B. these are from one female)	*217 (210 in good condition)*	avg. 289 SD 62 (100 pupae)
sex of pupae	*all male*	
H. zea Females (Stoneville) X H. assulta males (Thailand)		
larvae collected	*510*	
larvae dead (2/9/88)	*044*	
larvae pupated	*58*	avg. 365 SD 50 (58 pupae)
sex of pupae	*22 females 36 males*	
H. zea X H. zea (Stoneville colony)		avg. 361 SD 83 (100 pupae)

These data from Stoneville records. All records in quarantine facility.

Neal R. Spencer
406-482-2020
October 23, 1989

United States Department of Agriculture
Agricultural Research Service
Mid South Area
Southern Field Crop Insect Management Laboratory
P.O. Box 346
Stoneville, Mississippi 38776

February 7, 1990

SUBJECT: Genetic Sterility in Heliothis

TO: M. E. Degrugillier
Research Entomologist
USDA, ARS
Fargo, North Dakota

FROM: D. D. Hardee
Laboratory Director

I recently read your paper in Volume 54 of the Journal of Invertebrate Pathology on possible cause of sterility in Heliothis. This paper stimulated me to review again a letter from Neal Spencer (copy enclosed) which I received last October.

I would appreciate your "bottom line" comments on this subject - what is our next step, what proof do we have that VLP's in spermatocyst cells are contributing to sterility, would cooperative efforts between our two laboratories be worthwhile, etc.

In my opinion, we have reached a serious crossroad in this research effort in that we have two major obstacles in proceeding past St. Croix in evaluating genetic sterility: (1) cost of rearing is a major barrier for evaluating its potential in a large enough area to make the test scientifically acceptable; and (2) some of our critics contend that until we achieve genetic sterility in Heliothis zea that the dollars should not be spent on H. virescens. We have reduced cost of rearing significantly, but much more still needs to be done. To date we have made no progress with the latter - all crosses we have made appear to be morphologically impossible. We still have several species with which to attempt matings, but most of these are very exotic and collection is difficult and expensive.

I am not certain that I fully understand the ramifications or significance of your findings, and I would appreciate your evaluation from a practical standpoint. Marion Laster and others have come too far for us to back off now, but future research progress has been slowed drastically by a shortage of funds. I am open to your suggestions and comments.

Enclosure

cc:(w/enclo.)
J. R. Coppedge
R. M. Faust
M. L. Laster
D. C. Zimmerman

United States Department of Agriculture
Agricultural Research Service
Northern Plains Area
Red River Valley Agricultural Research Center
Biosciences Research Laboratory
P.O. Box 5674
State University Station
Fargo, North Dakota
58105

March 1, 1990

SUBJECT: Virus-like Particles and Backcross Sterility in Heliothis

To: D. D. Hardee
Laboratory Director
USDA, ARS
Southern Field Crop Insect Management Laboratory
Stoneville, Mississippi

From: M. E. Degrugillier
Research Entomologist

The following contains some of my thoughts on backcross sterility and the presence of virus-like particles (VLP's) in Heliothis in response to your letter of February 7, 1990. First of all, we have not proven, nor will we be able to prove utilizing electron microscopy, that the VLP's cause sterility. This must await the isolation and identification of the virus and incorporation into a suitable host so that it mimics the effects on sperm cells that we see in backcross males. We have begun the isolation procedure and it appears that we will be able obtain suitable quantities quite readily. Determining if it is a RNA or DNA virus is quite simply done, but the rest of the procedure would require considerably more time.

I will provide you with some of our findings, some of which tend to support the microbial theory as the basis of sterility. In addition I hope to provide some indication of their significance and where we can go from here.

Some of our observations include the following:

1. There is a close correlation between the presence of VLP's in spermatocyst cells and deformed sperm cells. In 6 day old adult H. virescens, 70% of sperm scored as abnormal (swollen or hollow mitochondria) had VLP's in the cyst cell cytoplasm and/or nucleus (73% if just nuclear VLP's are included). In H. subflexa, 64% of abnormal sperm had VLP's in the cyst cells (67% if only nucleus is included). In backcross testes, the correlation is 90% (92.5% for nucleus only). In 8-10 day old adults, the relationship was even more evident, ranging from 90-100% for the 3 groups. Conversely, in sperm bundles that were scored as normal, only up to about 5% had VLP's in the cyst cells.

March 3, 1950

SUBJECT: Virus-like Particles and Bacteriophage Sterility in Helicobacter

D. D. Ward,
Laboratory Director

Southern Field Crop Insect Management Laboratory
Stoneville,

2. The VLP's appear to be ubiquitous in Heliothis. The following species and strains have been examined. A minimum of 5 testes/group was tested unless otherwise stated.

a.	H. virescens	(Fargo colony)
b.	H. virescens	(Stoneville)
c.	H. subflexa	(Fargo)
d.	H. subflexa	(Clemson, SC)
e.	Backcross	(Fargo, 150-200th generation)
f.	Backcross	(Stoneville, ? ")
g.	F1's	(Tifton, GA) - 3 testes - (H. virescens males X H. subflexa females - Parent species captured from wild populations.
h.	F1's	(Fargo) group (a) above crossed with (d)
i.	H. zea	(Stoneville) - 3 testes examined
j.	H. assulta	(Stoneville) - 1 testis (Pakistan origin)
k.	F1's	(Stoneville) - group (i) crossed with (j) 2 testes examined
l.	H. punctigera	(Australia)
m.	" armigera	(Australia)
n.	F1's	Reciprocal Cross - Group (a) females crossed with (d) males

VLP's have been found in all of the groups listed above. In addition, they have been found in every testis examined to date from each of the groups. We will be receiving some testes of H. peltigera and H. armigera shortly from Israel, and some H. obsoleta from Bulgaria in the future.

3. Expression of the VLP's varies among different colonies of the same species or colonies of the same strain. Large crystalline arrays of VLP's were routinely found in cyst cells of our Fargo population of virescens and backcross colony, but they were never found in the Stoneville virescens or backcross colonies, even though the Fargo colonies originated from Stoneville some 10 years ago. I suspect that since our Fargo colonies are highly inbred, this may be the reason. These arrays were not found in any of the other species examined.

4. Expression of the VLP's varies among species as to the cell or tissue type in which they are found. The VLP's in virescens, subflexa, and backcross testes seem to be confined to eupyrene cyst cells: those from other species can be found in follicle cell nuclei, tracheal cell nuclei, apyrene spermatocysts, epithelial cells, etc.

5. Expression of the VLP's is age dependent in the adult. The older the adult, the higher the percentage of spermatocysts with VLP's.

6. VLP's are associated with heterochromatin in cyst cell nuclei. The particles often appear to be embedded within the heterochromatin clumps, and large masses of particles in grape-like clusters often lie adjacent to the clumps.

7. Eupyrene sperm from F1 testes are normal looking except that they are double-tailed. The mitochondrial derivatives (MD's) are not swollen or hollow and only low levels of VLP's are found. Backcross sperm from later generations, however, are extremely distorted and high levels of VLP's are found in cyst cells. This was true of F1 testes from all four groups examined (g, h, k, and n above). We have not examined BC1 or BC2 generation testes yet (we have some prepared from group (h) above).

8. Sperm in backcross males appear normal until they reach the early to mid spermatid stage, and then begin to swell and become grossly abnormal. At about this time, membrane whorls with darkly staining pepper-like material are found among the sperm cells within a bundle, apparently originating from the cyst cell cytoplasm. These membrane whorls are intimately associated with the sperm plasma membrane, and short rod-like bodies enclosed within a loose membrane system with attached spherical dense particles are visible within the cytoplasm of the spermatid. These bodies can then be seen associated with the outer membrane of the mithochondrial derivative (MD), and finally within the MD itself. This is accompanied by swelling of the MD. In short, it appears that the mitochondrial derivatives are becoming "infected" with some material from the cyst cells. This happens in virescens as well as in the backcross, but the virescens MD's don't swell. This happens in the larval and pupal stages. I hope to have a paper out this summer describing this phenomenon in a lot more detail.

9. F1 testes have microorganisms present in addition to VLP's. These extremely numerous extracellular organisms are found in the lumen of the testes and are interspersed among the sperm bundles and degenerating sperm cells. They have been seen in every F1 testes examined to date (g, h, k, and n) and appear similar to rickettsia or cell wall-less bacteria. They are common in punctigera, armigera, and assulta, but have not been found in virescens, subflexa, or backcross males.

10. Although the VLP's appear only in adult moths, they may be present in immature stages but in a different form. The outer membrane surrounding the swollen mitochondrial derivative of backcross sperm of immature stages often has masses of osmiophilic, ribosome-sized partices that encircle the entire MD. In sperm of adult moths, VLP's occupy the same position and surround the MD. This indicates to me that

what we see in the larvae and pupae may be virus particles without the outer capsid, but in adults, the virus has formed the capsid, utilizing lipoprotein from the mitochondrial membrane. Both of these types of particles are seen associated with other membrane surfaces, not only those of the MD.

11. VLP's may be universally present in all lepidoptera. We have examined 3 other species of lepidoptera available in our laboratory (Mediterranean Flour Moth, Anagasta kuehinella; Sunflower Moth, Homoeosoma electellum; Banded Sunflower Moth, Cochylis hospes) and all testes examined had VLP's present. Two of these are in the Fam. Pyralidae, and one is in Fam. Cochylidae.

The above findings imply several things. First of all, the chromatin-associated or chromatin-originating particles suggest that this virus may be a hereditary virus that has been integrated into the hosts' DNA. Indeed, similar particles (morphologically identical) have been described in Drosophila melanogaster that are holomogous to the transposasble element copia. These particles appear in various tissues under stress conditions (aging, irradiation, tissue culture, association with melanotic blood tumors). The size and morphological appearance of the particles are strikingly similar to those of Heliothis. It has been suggested that the copia transposable elements are derived from retroviral proviruses, long since entrapped in host chromosomes.

If the observation (#8 above) that sperm MD's become infected during the spermatid stage can be proven, it may go a long way in describing how the sterility phenomenon works. The source of the membrane whorls from which the infectious agents appear to arise is unknown. However, it is known that spermatocyst cells, including the mitochondria and other organelles, degenerate and disappear before the sperm bundles leave the testes. We have micrographs demonstrating the degeneration of cyst cell mitochondria and some of them definitely have VLP's within their interior. It is possible that the membrane whorls are degenerating cyst cell mitochondria and that they are releasing the infectious agent that becomes incorporated into the sperm mitochondrial derivative.

Another interesting finding is that sperm in F1 testes from all 4 groups appears similar (G,H,K,N above). The bundles contain normal looking sperm (except that most are double-tailed) and few are abnormal with swollen or hollow mitochondrial derivative and few have VLP's. Later backcross males have deformed sperm and lots of virus. It was the opinion of Proshold and LaChance that the early sterility (generations 1-5) was due to different causes than that of later backcross generations. Perhaps the early sterility is due to double-tailedness and later the virus causes

sterility by producing abnormal sperm.

I would like to briefly give a hypothesis on how this virus may be involved in sterility in order to address your question on how genetic sterility may be achieved in H. zea.

If indeed a hereditary virus is present in the genome of all Heliothis species, it must have occurred a long time ago. Although the VLP's from various species appear similar, the RNA or DNA composition may vary among the species. Assuming that the virus causes production of nonfunctional sperm, the host species must have had some mechanism to overcome the effects of the virus or the species would surely have been eliminated long ago. One such way of negating the effects of the virus would be to delay its expression long enough so that males could produce functional sperm while young, and later after they have passed their reproductive prime, it would not matter if nonfunctional sperm are produced. This may be occurring in the species we have examined, as the amount of expression of the VLP's is age dependent in males. The host species has possibly developed a "suppressor" system originating from the nucleas that keeps the viral genes in check until the males have reached old age. According to Steve Miller at the Gainesville lab, suppressor systems have been described in other species, although I am not familiar with the literature.

Within any one species, if the "infective" agent is originating from the cyst cell cytoplasm (degenerating mitochondria?), then it would be of maternal origin. It would enter the sperm mitochondrial derivative but would not be expressed because of the mechanism described above. On the other hand, if the agent is from a "foreign" species, i.e. subflexa, and enters a sperm MD of virescens, the supressor system for this agent would be lacking, the virus would be allowed to be expressed, and sperm would degenerate. This may not be evident initially, as F1 males still contain 50% subflexa genes, but by the 5th-6th backcross generation, the genome is almost exclusively virescens and lots of degenerating sperm and virus should be present.

Getting back to the real world, it may not be all that important in interspecific crosses if there is sterility or not in the early backcross generations if the above hypothesis is correct. Indeed, in our crosses, we did get some fertility in the F1's and 40% of F1 males transferred at least some eupyrene sperm to the spermatheca. Although the majority of sperm in F1 males are double-tailed, there are also many that are single tailed and appear normal; perhaps this is where the fertility lies.

In the earlier crosses at Stoneville that were successful involving H. zea X H. assulta, I understand that F2 males showed high fertility before the colony was lost. Perhaps this was because only single-tailed (normal) sperm reached the

spermatheca. These sperm may represent only a small portion of those that were actually produced. If the above hypothesis involving the virus is correct, it may take up to the 5th generation or so before sterility is complete (the time at which the genome is almost exclusively that of the species being backcrossed to).

In short, I think that it would be worthwhile to try and make the above cross with zea again since you know that these 2 species are compatible. It would not be too expensive or time consuming and would give useful results. I would be happy to cooperate by monitoring at the EM level what is happening at the cellular level each generation. Unfortunately, we terminated our backcross line at the BC2 generation and dropped our subflexa colony because of monetary reasons so I don't know for sure when the virus starts to be expressed at high levels.

I agree that it may not be wise to undertake a large control program involving release of backcross males until the mechanism of sterility is better understood and control of zea can also be undertaken. According to my colleagues, once the virus has been isolated and purified the above hypothesis can be tested using molecular biology approaches. I cannot say how long this will take because of the funding shortage.

I hope I have answered some of your questions. Please feel free to contact me if you need specific information on any of the above points.

cc:
Don C. Zimmerman

APPENDIX F. Communications Relative to Russian Wheat Aphid Research

DNA studies of natural enemies of the Russian Wheat Aphid

Richard L. Roehrdanz - ARS, Biosciences Research Lab, Fargo, ND
in cooperation with
Robert L. Burton - ARS Plant Science Research Laboratory,
Stillwater, OK

Biocontrol of the Russian Wheat Aphid (RWA) using native or introduced parasitoids or predators is a very promising line of investigation in the management of this pest. A number of species are currently under investigation at Stillwater and elsewhere to determine their host preferences, behavioral traits and effective rearing conditions. For example, several geographical isolates of *Diaeretiella rapae* from the United States and around the world are in colonization. It is important to know the relationship between these potentially disparate populations especially between the native populations and those of foreign origin should one of the latter prove to be a prime candidate for release trials in this country. It has already been suggested that there could be several sibling species in the Diaeretiella complex. It would also be useful to know something about the phylogeography or the range of genetic variability across native populations of these insects. This could help determine whether the select release strain is likely to interbreed with particular native populations, which could dilute its effectiveness, or coexist as a discrete subpopulation.

The suggested approach is to examine DNA, the primary genetic material to characterize molecular genetic variants or sequences that may be used a markers to aid in identifying populations and in studying intraspecific and interspecific relationships. Initial emphasis would be on the mitochondrial DNA molecule, which, because of its rapid rate of evolution is well suited for intraspecific comparisons. Its pattern of maternal inheritance also makes it useful for studying maternal lineages and dispersal and it is apparently free from recombination events that can complicate interpretation of data from nuclear sequences. The studies would look for RFLPs or restriction site differences that could be diagnostic of a population and provide estimates of nucleotide sequence divergence. It would probably be useful to attempt to clone the mtDNA genome to facilitate sequence studies and provide a ready source of probe DNA for population comparisons. This work would complement both the isozyme studies being undertaken in Stillwater and Columbia, MO and traditional biosystematics work at other locations. The insect populations would be obtained and reared at Stillwater and the molecular genetics would be carried out in Fargo.

Estimated budget for continuing funds $50,000

United States Department of Agriculture
Agricultural Research Service
National Program Staff
Beltsville, Maryland 20705

March 29, 1990

SUBJECT: Russian Wheat Aphid FY90 HQ Fund Release

TO: R. Roehrdanz
Research Geneticist
BRL, Fargo, ND

THROUGH: R. Leopold, Acting Research Leader
BRL, Fargo, ND

D. Zimmerman, Center Director
NCSL, Fargo, ND

T. Army
Area Director
Northern Plains Area

W. Klassen W Klassen 3/30
Associate Deputy Administrator
Plant and Natural Resource Sciences

FROM: Richard S. Soper Richard S Soper
National Program Leader
Biological Control

Your proposal entitled "DNA studies related to Russian Wheat Aphid" has been approved for funding for two years as follows:

FY90	$25,000
FY91	40,000

Headquarters will transfer $25,000 from CRIS 0500-00028-001-00D to CRIS 5442-22000-005-00D to support this activity. Please coordinate this research with Dr. Steiner, Columbia, MO and Dr. D. Reed, Stillwater, OK.

cc:
D. Cole
E. Knipling
BC Matrix Team

1772H

Posted: Thu, Oct 11, 1990 8:05 AM EST Msg: JGJA-3046-5528
From: RSOPER
To: AD.NPA, LC.Stillwater, RSoper
Subj: Forwarded message (correction)

October 9, 1990

SUBJECT: DNA studies related to Russian wheat aphid

TO: R. Roehrdanz
Research Geneticist
BRL, Fargo, ND

THROUGH: K. Narang
Research Leader
Fargo, ND

THROUGH: D. Zimmerman
Center Director
NCSL, Fargo, ND

FROM: R. Soper
National Program Staff
Beltsville, MD

Please provide me a 2-3 page summary of your research activity on the above subject. We are prepared to release an additional $40,000 for FY91, pending Congressional action on the ARS budget. However, before making this release it is important to review progress. Please indicate how your research has been coordinated with Dr. W. Steiner, Columbia, MO, and Dr. D. Reed, Stillwater, OK, as requested. Also, please indicate which CRIS should receive these temporary funds.

cc:
T. Army
H. Brooks
R. Burton
R. Faust

United States Department of Agriculture
Agricultural Research Service
Northern Plains Area
Red River Valley Agricultural Research Center
Biosciences Research Laboratory
P.O. Box 5674
State University Station
Fargo, North Dakota
58105

October 20, 1990

SUBJECT: DNA studies related to parasites and predators of the Russian wheat aphid

TO: R. Soper
National Program Staff
Beltsville, MD

THROUGH: S. Karl Narang
Research Leader
Fargo, ND

FROM: R. Roehrdanz
Research Geneticist
Fargo, ND

This project constitutes an effort to find mitochondrial DNA (mtDNA) variants that can be used as molecular markers to distinguish parasites and/or predators of the Russian wheat aphid that have diverse Old World geographic origins. To help get the project underway, R. Burton recommended that I visit the laboratory in Stillwater. The two days spent there at the end of June proved quite valuable not only from the standpoint of establishing a working relationship with the researchers there, especially D. Reed and G. Puterka, but also in discussing the possible directions and options for the research.

It was decided to begin work with the tiny parasitic wasp, Diaeretiella rapae. This Old World species is apparently fairly wide spread in the USA, the result of previous introductions to combat other aphids. With the advent of the RWA problem, annual collection forays have been made to obtain new material from regions where the RWA occurs. D. Reed was maintaining a number of colonies of this species from which insects could be collected, frozen and sent to Fargo. However, the insect is truly tiny (10+/mg or 150-200 = mass of one boll weevil). The average size of the Stillwater colonies is 100-200 individuals and possibly less when the greenhouses become very hot in midsummer. The potential problem is that the available biomass may be insufficient for certain molecular manipulations. One standard way to investigate mtDNA variability is to purify the mtDNA from the same species, clone fragments of it and use the cloned fragments as labeled probes on Southern blots of total DNA extracts of much smaller samples. This is not possible with the small amount of material available. (A recent study of satellite DNA in another species of similarly tiny wasps used 10,000 individuals as starting material, Molecular Biology and Evolution 7:351-364. 1990.) Thus the primary options for obtaining data on mtDNA variability would seem to be the use of labelled probes

derived from other insect species or the use of the polymerase chain reaction (PCR) to amplify specific regions of the wasp mtDNA from small samples. Both options are being investigated. Current efforts, problems, and possible future directions for each option are discussed below.

The first insects received were collected and frozen in Stillwater and shipped to Fargo in late July. They arrived at room temperature with no way of determining what maximum temperature might have been reached. Total DNA extractions were carried out using 1, 2, 5, 10, and 25 insects in two different extraction protocols: 1) a modified Drosophila procedure being used in Stillwater for aphids and 2) a "CTAB" procedure being used in Fargo for boll weevils. The extracts were digested with EcoRI and run on agarose gels. Ethidium bromide staining of the gels did not reveal any DNA for the extracts of 1, 2, 5, and 10 individuals. For the 25 individuals a small amount of DNA generally less than 2 kb in size was detected. Southern blotting and hybridization to 32-P labelled boll weevil (Coleoptera) mtDNA or Drosophila (Diptera) mtDNA did not detect any mtDNA in the extracts. The small size of the total DNA suggests that degradation of the sample had probably occurred during shipping.

D. Reed then made arrangements with R. Deerberg (USDA-APHIS, Mission TX) to ship mummies directly to Fargo. These were received and 100 or more adult wasps were collected over a week and frozen at -80 C. Fifty individuals from this material were used in each of the two extraction protocols. The stained gels showed evidence of larger size total DNA, but the blotting and hybridization to weevil or fly mtDNA did not reveal any mtDNA sequences. The two most likely explanations for this are either that there is still not enough mtDNA in that number of insects to detect hybridization or that the Diptera and Coleoptera mtDNA used as probe do not have sufficient homology with the wasp (Hymenoptera) mtDNA to hybridize effectively.

The amount of DNA in the extracts might be increased by using more insects or finding alternative extraction protocols. The hybridization signal might be much better if the probe DNA were from a more closely related insect, i.e. another Hymenoptera. The obvious well studied Hymenoptera is the honey bee. In an effort to find clones of honeybee mtDNA (or any other Hymenoptera) contacts were made with A. Sylvester (USDA Bee Lab, Baton Rouge), D. Smith (U. Michigan), S. Cameron (Washington U.), M. Hoy (Berkeley), all actively involved in molecular genetic research with Hymenoptera. None of them had any Hymenoptera mtDNA clones nor were they aware of any in this country. D. Smith told me that D. Crozier in Australia has cloned part or all of the honeybee mtDNA. A request has been sent to Dr. Crozier to see if he would be willing to send the set of clones.

The polymerase chain reaction is a procedure that can give a million fold amplification of DNA sequences that are situated between two specific homologous 20-25 base pair primer sequences. The amplified PCR product (i.e. wasp mtDNA segment) could be cut with restriction enzymes if the product is large enough to contain several restriction sites. Smaller products would be candidates for complete nucleotide sequencing and comparisons. Taking advantage of this technological advance requires first,

perfecting the techniques in a system with predictable results before trying the unknown insects and second, identifying potential primer sequences. I have done preliminary PCR amplifications using control lambda DNA template and primers. Product amplification was achieved but several variable parameters need to be optimized. Since there is no sequence information available for the wasp, selection of possible primers will center on ones that have proven successful in more than one other species of insect. To this end, contacts I have made with S. Cameron and C. Simon (U. Conn.) have proven invaluable. Dr. Simon has already sent me sequence information on over two dozen primers that have been used to amplify mtDNA and even offered to send a small amount of whatever pair we decide might be best to try first, probably a set that spans part of the 12S and 16S ribosomal RNA genes. Dr. Cameron likewise has volunteered to share information on primer sequences that have worked for bumble bees and braconid wasps, both Hymenoptera.

It should be mentioned here that W. Steiner (ARS, Columbia) is examining D. rapae for allozyme mobility differences. Once baseline information is acquired, it will be important for some of the same collections to be examined by both approaches especially where one of the approaches has detected some intraspecific variation.

Finally, another point came out of my meeting with the researchers in Stillwater. Several species of predators have also been collected in Eurasia for release in this country. Most notable are the Coccinellid beetles (lady bugs). These might provide a useful backup position should standard DNA techniques be slow to provide data about the tiny wasps. They are much larger and are Coleoptera which offers the possibility of using the cloned Coleoptera mtDNA that I already have as hybridization probes. Thus, if there is interest in examining the genetic variability of these insects, it should be easier to adopt current methodologies. These insects are not being reared at Stillwater but may be available from the Beneficial Insects Quarantine Lab in Newark or the Aphis Biological Control Lab in Niles, Michigan.

I request that FY91 temporary funds for mtDNA studies on parasites of Russian wheat aphid be transferred to CRIS # 5442-24000-006.

cc:
D Zimmerman
T Army
H Brooks
R Burton
R Faust
W Steiner

APPENDIX G. Communications Relative to Coordinated Research to Determine the Geographic Origin of *Heliothis* spp. Dispersing from Indigenous Hosts and Habitats

United States Department of Agriculture
Agricultural Research Service
Mid South Area
Southern Field Crop Insect Management Laboratory
P.O. Box 346
Stoneville, Mississippi 38776

October 3, 1990

SUBJECT: Prospectus for Coordinated Research

TO: Dr. James R. Coppedge
USDA, ARS, NPL
Cotton Insects Research
Bldg. 005, BARC-West
Beltsville, Maryland 20705

THROUGH: D. D. Hardee ~~DDH~~ 10-4-90
Laboratory Director

FROM: D. E. Hendricks DEHendricks
Research Entomologist

Enclosed is a prospectus of a research project that was originated in early August, 1990 by ARS scientists. Initial planning, support, and specimen collections for this study were initiated by ARS personnel as an "in-house" effort. However, researchers at various state agricultural experiment stations have shown much interest and enthusiasm in this effort. They have already contributed live moth specimens so that baseline "fingerprinting" techniques can be established and so the genetic profiles of moths collected from several key geographic regions can be characterized.

We thought that you would be interested in the purpose and intent of this research project, and welcome your support and suggestons. Although Dr. Karl Narang and his team at Fargo seem quite capable of developing the baseline fingerprinting techniques, I'm certain we will need your support to successfully carryout a project of this magnitude.

Plans are to proceed, this fall, by shipping additional live moths collected from different geographic regions to ARS, Fargo, ND, so Karl can continue with development of techniques required to genetically characterize the moths. We hope that this type of methodology can be worked during the winter months.

We're always glad to hear from you.

cc:
K. Narang, Fargo, ND
J. D. Lopez, College Station, TX
P. D. Lingren, Lane, OK

From: D. E. Hendricks
SIML, ARS, USDA
P. O. Box 346
Stoneville, MS 38776

October 2, 1990

PROSPECTUS FOR COORDINATED RESEARCH TO DETERMINE THE GEOGRAPHIC ORIGIN OF *Heliothis* spp. DISPERSING FROM INDIGENOUS HOSTS AND HABITATS.

Coordinators (ARS, FY 1990): Karl Narang, RL, Fargo, ND
Don Hendricks, Stoneville, MS
Juan Lopez, College Station, TX

Project Origin: U.S. Dept. Agriculture, Agr. Research Service, (In-House).
Research Period: July, 1990 to July, 1994.

Objective: Develop methods to identify the geographic origins of *Heliothis* spp. moths dispersing from unique habitats.

Research Approach: Establish baseline techniques to "fingerprint" *Heliothis* adults that have been collected alive from traps and/or adults reared from larvae collected from identified native or cultivated host plants. Fingerprinting techniques must be established for the adult (moth) stage, since candidate migratory specimens can be collected most expediently in the future as moths caught alive in traps baited with appropriate attractants.

Baseline fingerprinting will be established by a multiple approach; 1) by determining the genetic structure of moths collected from different geographic regions, and 2) by determining diagnostic enzyme loci and significant allel frequency differences. Genetic structure of the moths will be characterized by the biochemical patterns exhibited from analyses of isoenzyme DNA, mitachondrial DNA, ribosomal DNA, and hydrocarbon profiles.

Determine the origin of moths collected during subsequent years in different geographic regions by comparing their fingerprints with the baseline fingerprints of moths previously collected in these designated regions.

Prospectus for coordinated research (Con't):

Research Requirements: To establish the baseline fingerprints of moths from each geographic location, representative samples of living moths must be collected at locations within the geographic range of habitat supporting native individuals. Specimens may be collected as living adults by hand, or from exsisting or new traplines, or collected as last stage larvae from identified host plants (indigenous or cultivated) and allowed to develop to the moth stage. These specimens should be collected at intervals of 3 to 4 weeks throughout the period when host plants are available, possibly from April to November.

Specimens collected from native host plants and reared to the adult stage may provide the most reliable genetic profile for use as baseline fingerprint standards.

Living moths can be shipped by Express (overnight) Air Freight to Dr. Karl Narang, ARS, Fargo, ND for genetic fingerprint and biochemical analysis (July 90 - Dec 90).

Collection of Moths to Oct 90: Shipments of moths to ARS, Fargo, ND have been made during Aug, Sept, Oct, 1990 by ARS and various State Experiment Station researchers. Geographic locations of these moth collections and shipments are as follows:

ARS, Stoneville, MS
ARS, Oxford, NC
ARS, Lane, OK
ARS, College Station, TX
Bossier City, LA
McGehee, AR
St. Joseph Parish, LA
Ankeny, IA
Columbia, MO
Lubbock, TX

This research project has been coordinated within ARS using "in-house" support. Success will depend on the continued cooperation and help from both ARS and State researchers and technical personnel. Rate of progress may be limited by budgetary constraints.

APPENDIX H. Poster Titles/Scientists, Entomology Program Review, August 14-16, 1989

RED RIVER VALLEY AGRICULTURAL RESEARCH CENTER
BIOSCIENCES RESEARCH LABORATORY
FARGO, ND

BUILDING 1
SECOND FLOOR

LOCATION	SCIENTIST	POSTER TITLE
Rm. 283	ADAMS	Housefly Reproduction: The Role of Neuropeptides/Hormones
Rm. 297	BUCKNER	Excretion Mechanisms for Lepidopteran Insects
Rm. 215	GASSNER	Magnetic Resonance Imaging Research in Entomology
Rm. 258	HEILMANN	Isolation and Characterization of Sex-Specific cDNA and Genomic Clones of the Boll Weevil, Anthonomus grandis
Rm. 220	LEOPOLD	1) Bioregulation of Microfibril Orientation in Insect Cuticle 2) Ornithine Decarboxylase Gene Expression in Developing Embryos of the Housefly, Musca Domestica L.
Rm. 285	NELSON	Navel Very Long-chain Methyl-branched Hydrocarbons in Lepidopteran Pupae
Rm. 208	NORTH	Suppression of Natural Populations of Boll Weevils through Genetic Engineering
Rm. 292	POMONIS	Insect Cuticular Lipids
Rm. 273	ROEHRDANZ	Mitochondrial DNA Diversity Among Populations and Closely Related Species of Economically Important Insects
Rm. 274	ROJAS	Cryobiology and the Preservation of Germplasm of Insects

RED RIVER VALLEY AGRICULTURAL RESEARCH CENTER
BIOSCIENCES RESEARCH LABORATORY
FARGO, ND

BUILDINGS 5 AND 6
FIRST FLOOR

LOCATION	SCIENTIST	POSTER TITLE
Bldg. 6	DEGRUGILLIER	Latent, Hereditary Viruses of Heliothis: The Basis of Inherited Male Sterility?
Bldg. 5	McDONALD	Genetic Studies of Diabrotica Pests of Corn
Bldg. 6	RIEMANN	Lepidopteran Biology

BUILDING 1
FIRST FLOOR

LOCATION	SCIENTIST	POSTER TITLE
Rm. 153	BARKER	Sunflower Insect - Sunflower Interactions
Rm. 162	STILES	Cuticular Proteins of the Cotton Boll Weevil

SCIENTIST	POSTER TITLE
CHARLET	Host Plant Resistance and Management Systems for Sunflower Insects

APPENDIX I. USDA Research Priorities for Fiscal Year 1992 (Joint Council on Food and Agricultural Sciences)

Joint Council on Food and Agricultural Sciences

Secretariat:
Suite 302 Aerospace Building
U.S. Department of Agriculture
Washington, D.C. 20250-2200

June 30, 1990

Honorable Clayton Yeutter
Secretary of Agriculture
Washington, DC 20250

Dear Mr. Secretary:

As agriculture enters the decade of the 1990's, it is faced with many changes: new political and trade boundaries; a turbulent business environment arising from increased integration of the agricultural sector into the national and world economies; advances in technology; projected modifications in Government regulations; increased concerns about the environment, nutrition, and food safety; and potential shifts in weather patterns.

These complex, worldwide, multidimensional changes have serious implications for U.S. agriculture. They require the Nation's food, fiber, and agricultural industries, including small firms and family farms, to remain highly efficient and internationally competitive while providing wholesome products in an environmentally sound manner.

This report, *Fiscal Year 1992 Priorities for Research, Extension, and Higher Education*, represents the Joint Council's assessment of what the food and agricultural science and education system needs to accomplish in order to optimize the Nation's agricultural enterprise.

The five priorities for fiscal year 1992 are of equal status. They encompass the issues of:

- Compatibility and Sustainability of Agriculture and the Environment
- Global Competitiveness and Expansion of Agricultural Markets
- Abundant, Affordable, Safe, and Nutritious Food for Optimal Health
- Scientific and Professional Expertise in the Food and Agricultural Sciences
- Social Stability and Changing Values in America.

The Joint Council appreciates the opportunity to discuss this report with you.

Sincerely,

Charles E. Hess

CHARLES E. HESS
Cochair

H. Rouse Caffey

H. ROUSE CAFFEY
Cochair

Compatibility and Sustainability of Agriculture and Environment:
The well-being of the American people depends on natural resource endowments, including productive soils, forests, and rangelands and the diverse plants, animals, and microorganisms that inhabit them. These resources must be conserved and their productive capacity assured to sustain present living and environmental standards for future generations.

Ambitious agendas are required to address agricultural productivity and environmental protection as mutually compatible objectives. This priority addresses the need to:

- Understand the impacts of global climate change;
- Protect and improve water quality and quantity;
- Enhance safe and effective control of agricultural and forest pests and diseases;
- Sustain natural resource productivity; and
- Address issues of solid and hazardous waste.

Global Competitiveness and Expansion of Agricultural Markets:
The American food and agricultural system is the world's largest commercial industry, with assets of approximately $1.4 trillion. It accounts for 17 percent of the U.S. gross national product and employs more than 20 million people.

The ability to compete in domestic and foreign markets is key to the economic viability and continued prosperity of the agricultural sector and the Nation. To achieve this, the agricultural community must:

- Develop sustainable production and marketing systems that are economical and environmentally compatible;
- Improve production of food and fiber through germplasm collection, maintenance, and enhancement;
- Develop new processing technologies that add value to food and non-food products; and
- Improve animal health and welfare.

Abundant, Affordable, Safe, and Nutritious Food for Optimal Health:
The American consumer is the ultimate beneficiary of agricultural research, extension, and education. The modern miracle of U.S. agricultural productivity has resulted in a high-quality, low-cost food supply for Americans that is unequaled worldwide.

Vigorous research and education programs are required to continue to improve the quality and safety of food from farm to consumer and also to advance the understanding of how diet affects health and enhances the quality of life. This priority examines the need to:

- Ensure the safety and stability of consumer foods; and
- Provide optimal health through improved nutrition.

Scientific and Professional Expertise Base in the Food and Agricultural Sciences:
For a century this Nation has led the world in developing science and applying advancements in technology on behalf of the food and agricultural system. Predictions suggest that food and agricultural scientists also will lead the way in achieving breakthroughs in genetic engineering.

Bold initiatives are required to ensure that a well-trained cadre of highly qualified scientists and educators can meet the many challenges proffered in the global arena. This priority targets the need to:

- Stimulate development of scientists and professionals; and
- Ensure access to quality education.

Social Stability and Changing Values in America:
Youth, families, and communities are striving to adapt to and determine directions of the economic, social, and cultural changes taking place across the Nation and around the world.

The challenge to the science and education system is to assist individuals, families, and communities to develop their potential; assist them to find solutions; and help them apply their knowledge and skills to individual or local situations. The intent of this priority is to:

- Emphasize programs to assist youth at risk;
- Build support systems for individuals, families, and communities; and
- Revitalize rural America.